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PACHYTENE ANALYSIS OF STANDARD FRAGMENTS AND LARGE ISO-FRAGMENTS IN RYE

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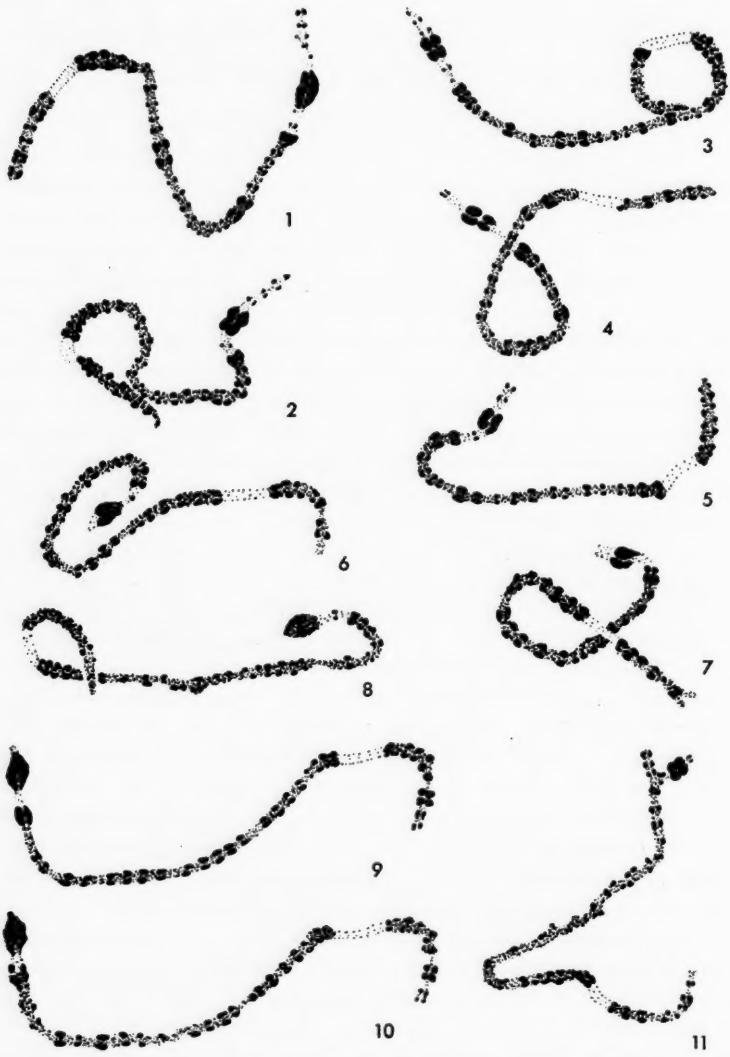
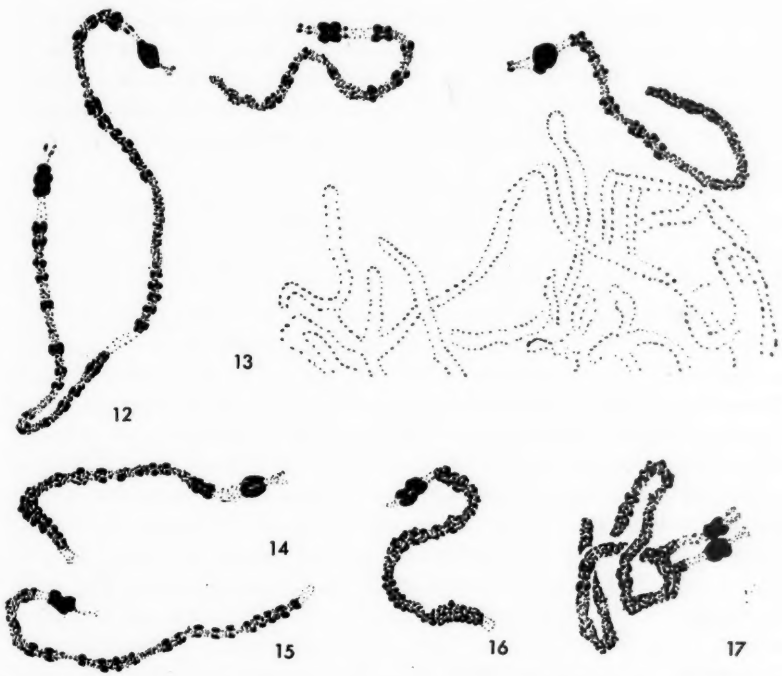
I. INTRODUCTION.

In rye, *Secale cereale*, most plants have $2n = 14$ in the root tips, but in various populations there may occur a minority of plants which in addition to the 14 normal chromosomes (the A chromosomes) contain accessory chromosomes (B chromosomes) of a special kind. The usual type of B chromosome is the so-called »standard fragment», which is characterized by a subterminal position of the centromere, separating a short headlike arm from the longer arm. The A chromosomes, on the contrary, have median or submedian centromeres. The standard fragment has about half the size of an A chromosome and, hence, at mitosis it may easily be distinguished by size as well as morphology (*cf.* MÜNTZING, 1944, Figs. 6—7). At meiosis the standard fragment, as well as its derivatives, never pairs with the A chromosomes. If two standard fragments are present they may form a small bivalent or appear as univalents. The most peculiar property of the standard fragment is its ability of numerical increase by non-disjunction. This process regularly occurs at the first pollen mitosis and at the corresponding stage in the ovules. In spite of this increase mechanism standard fragments only occur in a minority of the plants in a population, and in most cases only 2 such B chromosomes are present. This apparent contradiction is explained by the fact that, on an average at least, the B chromosomes have deleterious effects on vigour as well as fertility. Thus, there is a balance in the population between tendency to increase by non-disjunction and elimination owing to the bad genetical effects of these chromosomes. In some varieties meiotic elimination may also play a rôle. A summarizing report of these investigations was given by MÜNTZING (1949). In this review reference is given to a number of detailed reports published in Hereditas during the years 1943—1948.

In our cultures new types of B chromosomes have appeared, some of them presumably arisen by misdivision of the centromere (MÜNTZING,



Figs. 1—11. Pairs of standard fragments at pachytene in plants having $2n = 14 + 2$ standard fragments. — Figs. 1—5, material of Vasa II. Figs. 6—11, material of Östgöta Gråråg. Figs. 1—10, normal pairing. Fig. 11, a pairing abnormality indicating a deletion or duplication. — × 2700.



Figs. 12—17. Large iso-fragments at pachytene in a plant with $2n = 14 + 2$ large iso-fragments. — Fig. 12, the two iso-fragments are paired in their entire length. Fig. 13, the two iso-fragments do not pair with each other but show inter-arm pairing. Fig. 17, two iso-fragments probably showing a combination of inter-chromosome and inter-arm pairing. — × 2700.

1944). This process has resulted in the so-called large and small iso-fragments, the former probably corresponding to twice the long arm of the standard fragment, the latter to twice the short arm.

New possibilities for the study of A as well as B chromosomes in rye have been obtained by the pachytene technique recently developed by one of us (LIMA-DE-FARIA, 1948 and 1949). Thus, it has not only become possible to distinguish special chromosomes at pachytene but also to study them in great detail. The standard fragments are especially suitable for such studies, as they are smaller than the A chromosomes and easier to identify and follow in their entire length.

The present report is based on pachytene studies of standard fragments from two different varieties, Vasa II and Östgöta Gråråg, and of

large iso-fragments derived from the standard fragment of Östgöta Gråråg. The technique used was described in detail by LIMA-DE-FARIA (1948). The main features of this technique are fixation in acetic alcohol and staining in iron-aceto-carmine under application of pressure and heat. The slides are made permanent, using balsam as a mounting medium.

II. OBSERVATIONS.

1. STANDARD FRAGMENTS IN VASA II AND ÖSTGÖTA GRÅRÅG.

A preliminary description of the standard fragment in Vasa II was given by LIMA-DE-FARIA (1948). In the main these observations were confirmed by the present study, though some regions were found to be more variable than was first assumed. Figs. 1—5 show the appearance of the standard fragment of Vasa II or, more correctly, the pairs of standard fragments in plants having $2n = 14 + 2$ s. f. Figs. 1—2 are from the same plant, Figs. 3—5 from a sister plant. These individuals were not pure Vasa II plants but had been obtained by crossing an inbred line without B chromosomes as female parent with a Vasa II plant carrying 2 standard fragments. Thus, in spite of the hybridization the fragments are both derived from the Vasa II parent and even from the same chromosome, both chromatids of this chromosome passing to the generative nucleus at the first pollen mitosis. From Figs. 1—5 the following features may be seen:

(a) The centromere is clearly visible as an almost unstained region, generally without a clear chromosome structure. Only in slides with rather deeply stained chromosomes could a special chromomeric structure of the centromere be distinguished (LIMA-DE-FARIA, 1949).

(b) The centromere divides the chromosome into two arms of rather different size, which correspond very well to the long and short arms observed in somatic chromosomes of this kind.

(c) The long arm has a subterminal knob which is quite conspicuous. At least the main part of this knob is composed of 2 pairs of big chromomeres (*cf.* Figs. 2—4).

(d) The long arm is terminated by a short segment outside the knob. In this segment a maximum of 4 or 5 quite small pairs of chromomeres was observed. The smallness of the chromomeres apparently makes this segment more fragile and variable than parts having larger chromomeres.

(e) On the other, proximal side of the knob there is also a short,

thin and almost unstained »neck» segment, only containing about 2 pairs of chromomeres. Thus, on both sides of the heavily stained knob there are short sectors of quite an opposite appearance.

(f) The rest of the long arm (between the centromere and the region just mentioned) is not homogeneous with regard to size and staining intensity of the chromomeres. The part situated closest to the centromere generally shows a quite pronounced staining, and the same is true of the part close to the thin region mentioned under (e). There are also some conspicuous groups of big and markedly stained chromomeres in other parts of the long arm, but we have not yet sufficient information about their position and constancy.

(g) The short arm is rather markedly stained, especially the region closest to the centromere.

In the best cases, such as the one represented by Fig. 1, the chromomeres are distinct enough for a rather accurate count of their number. The short arm seems to be composed of 6—8 chromomeres. In the long arm the knob and the two surrounding »thin» segments comprise a maximum of about 9 chromomeres. In the rest of the long arm about 27 chromomeres may be distinguished. Thus, the total number of the long arm would be about 36. This, of course, is a relatively rough estimation, as the smallest chromomeres may be difficult to distinguish and the big ones may be compound. Anyhow, already the present preparations no doubt allow an estimation of the number of chromomeres.

Figs. 1—5, showing the standard fragments in Vasa II, represent two plants, but also in three other sister plants the chromosome in question was found to have the same appearance. Unfortunately the material available from the other variety, Östgöta Gråråg, is less representative, being derived from a single plant having $2n = 14 + 2$ s. f. The pachytene slides of this plant, however, were rather satisfactory, as is evident from Figs. 6—10, and probably give good information about the appearance of the standard fragment in Östgöta Gråråg.

A comparative study of the standard fragments in the two varieties (Figs. 1—5 and 6—10) revealed a rather close similarity. Also in Östgöta Gråråg there is a long and a short arm and a conspicuous knob in the terminal part of the long arm. Further similarities are represented by the thin neck-like region just proximal to the knob and by the presence of more deeply stained chromomeres on both sides of the centromere and close to the neck-like region just mentioned. In some cells the knob in Östgöta Gråråg was observed to be composed of 2 pairs of big chromomeres just as in the other variety.

A clear difference is met with in regard to the most terminal segment of the long arm. In Vasa II this segment was always more or less protruding and contained about 4 or 5 pairs of small chromomeres. In Östgöta Gråråg it was found to be much less conspicuous and sometimes, as in Fig. 8, it seemed to be completely absent. No clear chromomeric structure could be distinguished.

Other differences probably occur in the short arm. In Östgöta Gråråg a subterminal constriction was almost regularly present (*cf.* Figs. 6, 7, 9, 10), and the very end of the arm often appeared to be rather unstained and devoid of clearly visible chromomeres. Sometimes the end also appeared to be bifurcated (*cf.* Figs. 7, 10), the two homologues not being closely paired for a short distance. All these features appeared to be absent in Vasa II, the short arm here having a more uniform appearance (Figs. 1—5).

Fig. 11 shows a pairing abnormality in the standard fragments of Östgöta Gråråg. Quite similar pictures indicating a deletion or duplication were seen in some more cells of the same slide, and, thus, this irregular pairing may have a special though as yet unclear significance.

2. THE LARGE ISO-FRAGMENT.

Rather good pachytene slides were obtained from an individual having $2n = 14 + 2$ large iso-fragments. This plant belonged to the variety Östgöta Gråråg, the large iso-fragment presumably representing twice the long arm of the standard fragment of this variety. This was, indeed, verified by a study of the pachytene configurations. In most cells the two iso-fragments were present as two separate bodies as shown by Fig. 13. These bodies strikingly resemble the long arm of the standard fragment (Figs. 6—10). The size is about the same and the characteristic knob is present. It is again separated from the major part of the chromosome by a thin »neck»-region. The most terminal part outside the knob was found to be somewhat more developed in the iso-fragment (Figs. 12—17) than in the corresponding standard fragment (Figs. 6—10), but it is doubtful whether this apparent difference is significant. More probably it is accidental and caused by slight differences in the success of fixation or staining between the few slides so far available. At any rate the general similarity between the long arm of the standard fragment and the structures seen in Figs. 13—16 is quite convincing. In these cases the two identical arms of the standard fragments have evidently made »fold backs» and are paired with themselves. This means that the centromere must be sharply bent and,

hence, less visible than in the standard fragments (Figs. 1—11). In Fig. 13 the centromere of the intra-paired iso-fragments could not be distinguished, but in Figs. 14—16 it is clearly visible as a short terminal segment without chromomeres. Figs. 14—16 are from cells in which only one of the iso-fragments could be distinguished, the other one being entangled somewhere among the long A chromosomes.

In some cases the inter-arm pairing is replaced by an inter-chromosome pairing, the two large iso-chromosomes being paired with each other in their entire length. A pachytene bivalent of this kind is represented by Fig. 12. The centromere is evidently strictly median, and the two arms are mirror images of each other. The typical characteristics of the long arm of the standard fragment are clearly visible in both arms of the pair of iso-fragments. In the first place the terminal knobs with their necks and appendages attract attention. The densely stained regions near the centromere and near the necks may also be distinguished.

In one case (Fig. 17) the large iso-fragments paired in a way which is probably intermediate between the two usual alternatives represented by Figs. 12 and 13. This case probably represents a mixture of intra- and inter-chromosome pairing, four free ends being visible.

III. MEASUREMENTS.

1. COMPARISON BETWEEN THE STANDARD FRAGMENTS OF THE TWO VARIETIES.

Owing to the pressure used when making the slides the pachytene chromosomes observed were quite flattened and hence quite suitable for measurements. Such measurements were undertaken on the drawings with the help of a map measurer. In the standard fragments the short arm, the centromere and four different regions of the long arm were measured separately. The data obtained are given in Table 1. The different regions of the long arm, denoted by the figures 1, 2, 3 and 4 in the table, may be defined as follows (*cf.* Fig. 1). Region 1: the main part of the long arm from the centromere to the »neck»; Region 2: the faintly stained neck region with small chromomeres just proximal to the knob; Region 3: the knob; Region 4: the faintly stained appendage distal to the knob.

The data in Table 1 are based on a total of 19 cells from Vasa II and of 9 cells from Östgöta Gråråg. The 19 cells of Vasa II represent

TABLE 1. *Size and variability of different regions in standard fragments of two rye varieties.*

Variety	Region	Average	*n*	Variance	Standard deviation	Coefficient of variation (v)	Standard error of v
Vasa II	Short arm	24,16	19	7,70	2,78	11,51	1,92
» »	Centromere	9,95	19	11,50	3,39	34,07	6,81
» »	Long arm 1	85,11	19	81,66	9,04	10,62	—
» »	» » 2	6,21	19	2,06	1,44	23,19	—
» »	» » 3	6,68	19	1,23	1,11	16,47	—
» »	» » 4	9,79	19	7,51	2,74	27,99	—
» »	» » 1—4	107,79	19	95,29	9,76	9,05	1,49
» »	Total chromosome	141,89	19	170,54	13,05	9,20	—
Östgöta Gråråg	Short arm	25,56	9	4,03	2,01	7,86	1,87
» »	Centromere	10,78	9	4,20	2,05	19,02	4,82
» »	Long arm 1	85,33	9	45,25	6,73	7,89	—
» »	» » 2	5,44	9	5,11	1,67	30,70	—
» »	» » 3	6,00	9	1,00	1,00	16,67	—
» »	» » 4	1,89	9	1,11	1,05	55,56	—
» »	» » 1—4	98,67	9	36,00	6,00	6,08	1,44
» »	Total chromosome	135,00	9	63,00	7,94	5,88	—

Each unit corresponds to 0,25 μ.

5 different individuals (sister plants), the 9 cells of Östgöta Gråråg one individual.

According to the table, chromosome length was on an average 141,89 in Vasa and 135,00 in Östgöta. The significance of this difference was tested with an analysis of variance. This gave a v^2 of 2,11 and a P intermediate between 0,2 and 0,05. Thus, though the measurements indicate that the standard fragment at pachytene was longer in Vasa than in the other variety, this difference is not significant. Comparing the average values of each chromosome region separately, the *short arm* has the value 24,16 in Vasa and 25,56 in Östgöta. An analysis of variance gave a v^2 of 1,82 and a P again intermediate between 0,2 and 0,05. The *centromere* as well as *Regions 1*, *2* and *3* had rather similar average values in the two varieties, as may be seen from Table 1, and tests concerning the significance of the slight differences obtained were considered superfluous.

With regard to *Region 4*, on the contrary, the average values are

as different as 9,79 (Vasa) and 1,89 (Östgöta). A test of this difference gave a v^2 of 68,81 and a P smaller than 0,001. Thus, in the material observed the »appendage» distal to the knob was significantly longer in Vasa than in Östgöta. A rather significant difference was also found with regard to the total length of the long arm (regions 1—4), the average values being 107,79 in Vasa and 98,67 in Östgöta. In this case the v^2 value was 6,60 and P intermediate between 0,05 and 0,01.

For each chromosome measured the ratio between the lengths of the long and short arms was calculated. A statistical treatment of the values obtained showed this ratio to be different in the two varieties. In Vasa the ratio ranged from 3,81 to 5,18, with an average of 4,49. In Östgöta the corresponding range was from 3,41 to 4,29 with an average of 3,88. Testing this difference in the usual way, the following values were obtained:

	Degrees of freedom	Sum of squares	Mean square
Between series	1	2,32	2,3200
Within »	26	4,38	0,1685
Total	27	6,70	—

This gives a v^2 of 13,769 and a P smaller than 0,001.

2. THE LARGE ISO-FRAGMENT.

In the large iso-fragment the measurements of the centromere and the four regions corresponding to those of the long arm of the standard fragment gave the following results (Table 2).

TABLE 2. *Large iso-fragment. Chromosome length at pachytene.*

Chromosome region	Centromere	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 1–4 total	*n*
Iso-fr. paired with itself.....	(1,11)	75,56	5,33	5,67	4,78	91,33	9
Two iso-fragments paired	8,00	81,50	5,00	7,00	4,50	98,00	2

A comparison between these average values and those of the standard fragment of the same variety, Östgöta (Table 1), shows in the main a very good correspondence. In Östgöta the total length of the long arm was 98,67 units and in the iso-fragments showing inter-chromosome pairing (Fig. 12) the corresponding value was found to be 98,00. In the iso-fragments which were paired with themselves the length seems to be somewhat lower, the average value being 91,33. However, the difference 98,67—91,33 was tested with an analysis of variance

and was not found to be significant, v^2 being 3,57 and P intermediate between 0,2 and 0,05.

The general similarity in size and morphology between the arms of the large iso-fragment and the long arm of the standard fragment does not exclude some slight but significant differences between the two groups of material measured. Thus, Region 4, the appendage outside the knob, was longer in the large iso-fragment than in the corresponding fragment. The two average values of the large iso-fragment corresponding to the two modes of pairing were 4,78 and 4,50. In the standard fragment of Östgöta the corresponding value was only 1,89. The difference 4,78—1,89 is significant, v^2 being 29,34 and P smaller than 0,001.

On the other hand, Region 1 was found to be somewhat longer in the standard fragment than in the large iso-fragment. The average values to be compared are 85,33 (standard f.) and 75,56 (large iso-f. showing inter-arm pairing). Testing this difference in the usual way gave a v^2 of 6,60 and a P intermediate between 0,05 and 0,01.

Regions 2 and 3 evidently closely correspond in the standard fragment and the large iso-fragment. With regard to Region 2 the average values were 5,44 (st. f.), 5,33 (large iso-f., inter-arm pairing), 5,00 (large iso-f., inter-chromosome pairing). For Region 3 the corresponding values were 6,00, 5,67 and 7,00 respectively.

In the large iso-fragments showing inter-chromosome pairing the centromere seems to have about the same length as in the standard fragment ($\bar{x}$ being 10,78 and 8,00 respectively). In the large iso-fragments with inter-arm pairing the centromere was apparently much shorter, the average value being as low as 1,11. This value is uncertain, as the centromere was not always clearly seen. Anyhow, it reflects the fact that the centromere is folded as a consequence of the inter-arm pairing.

3. A COMPARISON BETWEEN THE VARIABILITY OF THE CENTROMERE AND THE OTHER CHROMOSOME REGIONS.

When supervising the results of the measurements it was realized that the centromere is more variable in size than the chromosome arms. This observation is supported by the following calculations, which also allow comparisons between the variability of the different regions of the chromosome arms. The basic data obtained are given in Table 1. Besides the average values this table also contains the values of variance (s^2), standard deviation (s), coefficient of variation (v) and, in some cases, the standard error of the coefficient of variation (e_v). The latter value

was calculated according to the formula $e_v = \pm \frac{v}{\sqrt{2n}} \cdot \sqrt{1 + 2\left(\frac{v}{100}\right)^2}$ (*cf.* KEMP, 1942; DAHLBERG, 1948). The simpler formula $e_v = \frac{v}{\sqrt{2n}}$ was first tried but gave e_v values deviating too much from those obtained with the more accurate formula.

In the first place it is of interest to compare the variability of the centromere with that of the long and short arms. Extracting the values necessary for this comparison from Table 1 the following data were obtained:

Variety	Vasa II ($n = 19$)			Östgöta Gråråg ($n = 9$)		
Part of standard fragment	short arm	centro-mere	long arm	short arm	centro-mere	long arm
v	11,51	34,07	9,05	7,86	19,02	6,08
e_v	1,92	6,81	1,49	1,87	4,82	1,44
Differences between v-values		22,56	25,02		11,16	12,94
e_v, diff.		7,08	6,97		5,17	5,03
t		3,186	3,590		2,159	2,573
P		0,01>P>0,001	0,001		0,05>P>0,02	0,05>P>02

Thus, in Vasa II the coefficient of variation is 11,51 for the short arm and 34,07 for the centromere. The difference between these values is 22,56. The standard error of this difference (e_v diff.) was calculated according to the usual formula $m_{Diff.} = \sqrt{m_1^2 + m_2^2}$ on the basis of the two standard errors of the coefficients of variation. Thus, in the present case e_v diff. is equal to $\sqrt{1{,}92^2 + 6{,}81^2} = 7{,}08$. The t-value is $\frac{22{,}56}{7{,}08} = 3{,}186$. For a total of $18 + 18 = 36$ degrees of freedom this value corresponds to a P value intermediate between 0,01 and 0,001 (BONNIER and TEDIN, 1940). In the same way a comparison between the variability of the centromere and the long arm of Vasa II gives a t-value of 3,590, which corresponds to a P of 0,001.

In the other variety, Östgöta Gråråg, similar results were obtained, though in this case the differences were less marked. Nevertheless the two t-values obtained (2,159 and 2,573) both correspond to P values smaller than 0,05.

From the v-values of Table 1 characteristic differences in the degree of variability of the different regions of the long arm may also

be seen. In Vasa II the variability is lowest in region 1 ($v = 10{,}62$), somewhat higher in region 3 ($v = 16{,}47$) and still higher in regions 2 and 4 ($v = 23{,}19$ and $27{,}99$ respectively). Exactly the same order 1, 3, 2 and 4 is repeated in the other variety, Östgöta Gråråg, the coefficients of variation for these regions being $7{,}86$, $16{,}67$, $30{,}70$ and $55{,}56$ respectively. In both varieties the most variable regions are the »neck» and the »appendage», which both appear fragile and have small and weakly stained chromomeres. The densely stained knob region and the remaining major part of the long arm, which has many distinct and relatively large chromomeres, show a lower degree of variability, and the same is true of the short arm, which is also well stained and contains several distinct chromomeres.

IV. DISCUSSION.

The most frequent type of accessory chromosome in rye, the standard fragment, may be easily distinguished from the other chromosomes owing to its smaller size and characteristic morphology. In somatic mitosis the short head-like arm appears to represent about one fifth of the total length of the chromosome (*cf.* MÜNTZING, 1948, p. 440) and approximately this proportion, 1 : 4, between the two arms was also observed at pachytene. In Vasa II the ratio between the length of the long and short arms was found to be $4{,}49$, the corresponding value in Östgöta Gråråg being $3{,}88$. The difference between these average ratios is significant. However, as the number of individuals involved in the measurements was very limited, it may be uncertain whether the established difference is representative of the varieties or only of the individuals used for collecting the data. However, the difference now found points in quite the same direction as previous significant results. Measurements of the length of the standard fragment in somatic mitosis have been made (MÜNTZING, 1945, p. 470) showing this chromosome to be about 11 per cent longer in Vasa II than in Östgöta Gråråg. Measurements of the diameter of unpaired standard fragments at first metaphase of meiosis were also undertaken (*l. c.*) and again showed the standard fragment of Vasa II to be significantly larger. According to the pachytene measurements (Table 1), the size difference is confined to the long arm and especially to the faintly stained appendage with small chromomeres distal to the knob. More material of both varieties is needed, however, before definite conclusions can be drawn as to the precise nature of the difference. Anyhow, the present pachytene results

support the previous observations of a length difference between the standard fragment of the two varieties and strongly suggest that this difference is chiefly caused by a special segment in the long arm. The short arm of the B chromosome in question had about the same length in the two varieties, but certain morphological details described above (p. 258) were different. It remains to be seen whether these morphological differences will recur when more material is studied.

In plants of Östgöta Gråråg having 2 standard fragments the pairing between these fragments at first metaphase of meiosis is often incomplete and strikingly different from the good pairing in the other variety (*cf.* MÜNTZING, 1945). The occurrence of such a difference is rather surprising, and the causes of the poor pairing of the standard fragments in Östgöta Gråråg are unknown. The present observations show that there is no difference in degree of pachytene pairing, the association of the two standard fragments apparently being complete in both varieties. This is, indeed, to be expected, as the pairing takes place between the two quite identical chromatids failing to separate during the first post-meiotic division in one of the parent plants. Consequently, the poor metaphase pairing of the standard fragments in Östgöta Gråråg must be caused by post-pachytene conditions, *viz.* a reduced chiasma frequency. As the size difference between the standard fragments of Vasa and Östgöta is rather slight, a genotypic control of the chiasma formation is probably responsible for the difference observed.

Though some slight differences between the standard fragments of the two varieties have been observed, their main morphology is quite the same. As already noted by LIMA-DE-FARIA (1948), these B chromosomes stain in the same way as the ordinary A chromosomes, and at this stage at least there is no conspicuous heterochromatic region which might explain the occurrence of the non-disjunction mechanism. The knob near the end of the long arm may be considered to be heterochromatic, it is true, but similar knobs are also present on the A chromosomes which do not show non-disjunction. The typical appearance of the standard fragment at the stage of non-disjunction (*cf.* MÜNTZING, 1946, p. 106) also shows that the segment mainly involved in the process is not the terminal part of the long arm but a more proximal segment. A good opportunity for a more detailed analysis is offered by a deleted fragment type that has lost a segment of the long arm and at the same time the ability of non-disjunction (MÜNTZING, 1948). A pachytene analysis of this chromosome, including chromomere counts, will pro-

bably give rather exact information concerning that part of the standard fragment which is responsible for the non-disjunction mechanism.

Among other things, the measurements undertaken demonstrated a greater variability in length of the centromere region than of the chromosome arms. This indicates a greater elasticity of the centromere and a greater rigidity of the chromosome arms. Further information about this question was obtained from the fact that two regions of the long arm, the »neck» and the »appendage», were found to be more variable in length than the remaining, main part of the arm. The neck and the appendage have few and quite small chromomeres, just as the centromere region. Hence, presence of chromomeres, staining normally, tends to give the chromosome sections carrying such chromomeres a greater rigidity than sections without or with few and small chromomeres. This is the same situation as in the salivary gland chromosomes, in which the dark bands show less elasticity than the unstained substance between the bands (BRIDGES, 1938).

In the standard fragment it is rather striking that the sequence of segments showing different degrees of staining is apparently not at random but shows a certain regularity. Thus, intercalary segments with poor staining are on both sides surrounded by segments with strong staining. This is true of the centromere as well as the neck region, and it should also be noted that the terminal and weakly stained appendage is situated close to the big and strongly stained knob. Starting from a supposed stage in which the substance taking the stain (the nucleic acid) is evenly distributed, a new stage is developed in which some regions apparently absorb more or less completely the staining substance present in both the adjoining regions.

Previous evidence strongly indicated that the so-called large iso-fragment represented twice the long arm of the standard fragment (MÜNTZING, 1944). The present pachytene analysis has verified this assumption at least as regards the main features of the long arm. One possible difference was observed, *viz.* the terminal appendage seemed somewhat longer in the large iso-fragment than in the corresponding standard fragment. It is rather probable, however, that this difference is only apparent and caused by a slight difference in the success of fixation or other environmental disturbances.

The mode of pairing at pachytene also verifies the supposed constitution of the large iso-fragment. In most cases in plants with $2n = 14 + 2$ large iso-fragments the two homologous arms of the same chromosome were intimately paired. Apparently this inter-arm pairing

is more frequent than inter-chromosome pairing, the two homologous large iso-fragments in the latter case being paired in their entire length. However, the latter mode of pairing is more difficult to distinguish at pachytene, and data from first metaphase are necessary to elucidate the frequency of the two modes of pairing. It is also possible that in some cases there is a combination of inter-arm and inter-chromosome pairing as indicated by one pachytene configuration (Fig. 17).

Studies of the metaphase pairing have not yet been made in plants with two or more large iso-fragments but observations of this kind were made in plants having a single large iso-fragment (MÜNTZING, 1944, Figs. 20—29). This chromosome often showed inter-arm pairing and appeared as a disc at first metaphase. Observations of earlier stages (diakinesis and diplotene) showed that the metaphase discs were really closed rings. The present observations on the appearance and pairing behaviour of the large iso-fragments at pachytene are evidently in perfect accordance with the previous results.

SUMMARY.

(1) Pachytene studies and measurements of some types of accessory chromosomes in rye (*Secale cereale*) have been undertaken, the material studied being so-called standard fragments and large iso-fragments. At pachytene these chromosomes have a characteristic morphology and may be easily distinguished from the other chromosomes. In favourable cases the number of chromomeres may be counted.

(2) Standard fragments of the varieties Vasa II and Östgöta Gråråg have essentially the same appearance, the main characteristics being the presence of a knob near the end of the long arm, a terminal appendage outside the knob and a thin neck region just proximal to the knob. The regions on both sides of the centromere and both sides of the neck are more deeply stained than the adjoining parts of the chromosome.

(3) The standard fragments of Vasa II and Östgöta Gråråg were found to show some slight but significant differences, which point in the same direction as previous observations on somatic chromosomes. The ratio between the long and short arm was greater in Vasa II than in the other variety, this difference being caused by the long arm, which was longer in Vasa than in Östgöta. This difference was mainly caused by the terminal »appendage». More material is needed to verify that

these and other small differences observed are representative of the varieties and not only of the material so far studied.

(4) By calculations of the coefficients of variation for different chromosome regions it was found that the centromere is more variable in length than the chromosome arms. In the long arm the »appendage» and »neck» regions, which have small and weakly stained chromomeres, are more variable than the other regions having larger and more numerous chromomeres.

(5) The supposed constitution of the large iso-fragment was verified by the pachytene analysis, this chromosome being composed of two identical arms representing the long arm of the standard fragment. In plants with two large iso-fragments inter-arm as well as inter-chromosome pairing was observed.

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X-RAY EFFECTS IN FRUIT TREES

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INTRODUCTION.

IN order to produce vegetative mutations experiments with X-ray treatment of apple scions were initiated in 1944 by Å. GUSTAFSSON and FR. NILSSON. The irradiation was carried out at the Radiophysics Institute, Stockholm, by Dr. A. FORSSBERG. In 1944 and 1945 the scions were grafted partly at the State Horticultural Research Station, Alnarp, and partly at the Balsgård Fruit Breeding Institute. The experiments were repeated in the following years, from 1946 also including pears and cherries. The material from the last years all belongs to the Balsgård Institute, where observations concerning the effect have been made under the direction of FR. NILSSON (till April 1, 1948) and I. GRANHALL (after this date) in co-operation with E. J. OLDÉN and BERTA DANIELSSON.

The experiments are included in the research series on induced mutations, directed by Å. GUSTAFSSON and defrayed by government grants and the Knut and Alice Wallenberg Foundation.

TREATMENT AND FIRST SEASON GROWTH OF SCIONS.

1944. — Still dormant scions of four apple varieties were cut early in the spring and then irradiated with 500, 1,500, 5,000, and 7,500 *r*-units. The varieties were Säfstaholm and Åkerö (local Swedish varieties), Cox's Orange and Gravenstein. These varieties were chosen because they already spontaneously give rise to vegetative mutations (sports). At Balsgård the scions were grafted on the clonal stock E.M.IV. A comparatively great lethality was found to have occurred among the treated scions (Table 1) when the number surviving was counted in the early spring of 1949. No habitual effects were, however, observed on the survivors during the summer. At Alnarp where E.M.IV was also used as stock, the percentage of survivors was higher. There are no notes of abnormalities in the first year.

Fig. 1. Knee and bifurcations in scions of Williams pear, 1948—5,000 *r*. — Fig. 2. Knee and double bifurcation in scion of Frogmore cherry, 1948—2,500 *r*.

TABLE 1. *Number of grafted (G) and surviving (S) scions from the trial of 1944.*

Apples (on E.M.IV)	Untreated		500 *r*		1,500 *r*		5,000 *r*		7,500 *r*	
	G	S	G	S	G	S	G	S	G	S
Balsgård:										
Säfstaholm	1	0	2	1	2	1	2	1	2	0
Åkerö	1	1	2	1	2	2	2	1	2	1
Cox's Orange	3	2	2	0	2	0	2	2	2	0
Gravenstein	1	1	2	0	2	1	2	0	2	0
Σ	6	4	8	2	8	4	8	4	8	1
% surviving	67		25		50		50		13	
Alnarp:										
Säfstaholm	2	1	3	2	3	1	3	2	3	2
Åkerö	1	1	3	3	3	2	5	3	3	3
Cox's Orange	1	1	3	3	3	2	4	0	3	1
Gravenstein	1	1	4	2	3	1	3	2	3	2
Σ	5	4	13	10	12	6	15	7	12	8
% surviving	80		77		50		47		67	

1945. — Only Cox's Orange and Gravenstein were treated. The strength of radiation was increased on account of the failing results of the preceding year, and 5,000, 10,000, 15,000, 20,000, and 25,000 *r*-units were applied. The Cox's Orange scions were grafted on an old tree (called »Tree R»), probably a chance seedling. The Gravenstein scions were worked on an old tree (»B») of the variety Oranie, but all

scions that had received 10,000 *r* or more died (Table 2). The survivors of Gravenstein 5,000 *r* showed no effect of the treatment, but in the case of Cox's Orange 5,000 *r* seemed to cause a slow and somewhat stunted growth, possibly a result of irradiation disturbances. No other effects appeared. Another portion of Gravenstein scions, treated with the same above-mentioned doses, were grafted on seedling stocks at Alnarp. There is still one tree from each dose surviving. In the first summer no special abnormalities were observed.

TABLE 2. *Number of grafted (G) and surviving (S) scions from the trial of 1945.*

Apples (on old trees):	5,000 *r*		10,000 *r*		15,000 *r*		20,000 *r*		25,000 *r*	
	G	S	G	S	G	S	G	S	G	S
Cox's Orange	10	5	10	0	10	0	10	0	10	0
Gravenstein	10	6	10	0	10	0	10	0	10	0
Σ	20	11	20	0	20	0	20	0	20	0
% surviving	55		0		0		0		0	

1946. — There were treated six varieties of apples (the diploids Boiken, Cox's Orange, Ellison's Orange, Pigeon, the triploids Gravenstein and Ribston), one of pears (Esperens Herre = Belle Lucrative),

TABLE 3. *Number of grafted (G) and surviving (S) scions from the trial of 1946.*

(1) *Apples* (on E.M.IV):	1,000 *r*		2,500 *r*		5,000 *r*		10,000 *r*	
	G	S	G	S	G	S	G	S
Boiken	6	3	5	0	5	0	6	0
Cox's Orange	5	4	5	2	5	0	6	0
Ellison's Orange	6	2	6	1	6	1	5	0
Pigeon	5	0	5	1	5	1	5	0
Gravenstein	5	1	6	1	6	0	6	0
Ribston	4	1	6	1	5	0	6	0
Σ	31	11	33	6	32	2	34	0
% surviving	39		18		7		0	
(2) *Apples* (double-worked):								
Boiken	4	4	5	2	5	2	5	0
Cox's Orange	5	4	5	5	5	0	4	1
Ellison's Orange	4	4	4	3	4	4	5	0
Pigeon	5	3	5	5	5	0	6	0
Gravenstein	5	5	4	4	4	0	4	0
Ribston	6	6	4	4	5	0	4	0
Σ	29	26	27	23	28	6	28	1
% surviving	90		85		21		4	

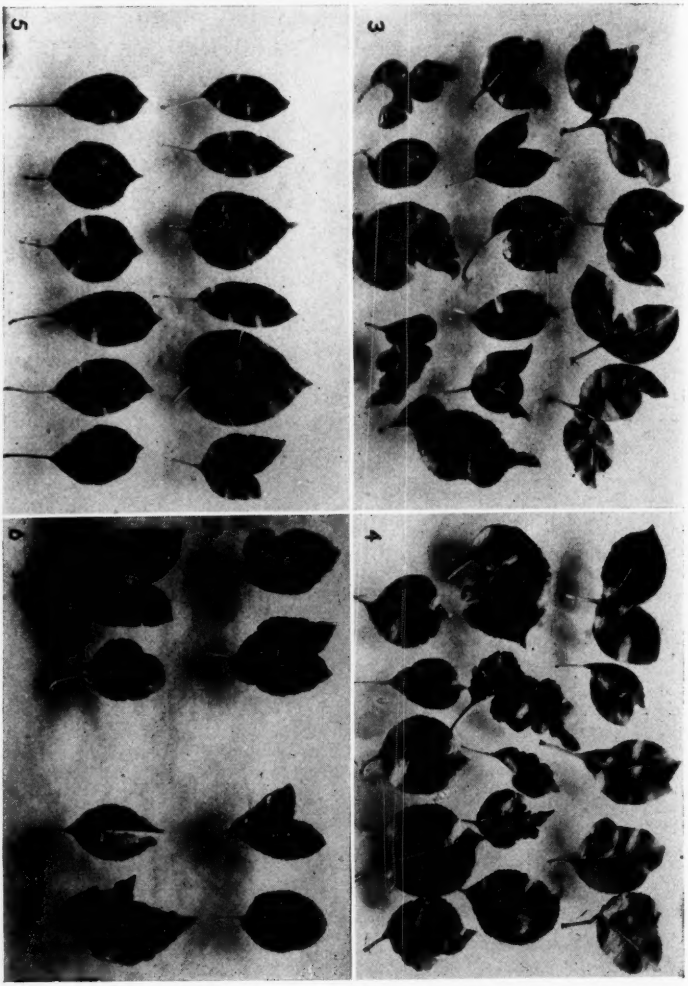

Fig. 3. Leaf deformations in Williams pear, 1948—5,000 *r*. — Fig. 4. Leaf deformations in Moltke pear, 1948—5,000 *r*. — Fig. 5. Leaves from untreated trees of Williams (upper row) and Moltke pear (lower row). Note the bifurcated leaf to the right in the Williams row. — Fig. 6. Leaf deformations in Cox's Orange apple, 1948—5,000 *r*.

	1,000 *r*		2,500 *r*		5,000 *r*		10,000 *r*	
(3) *Pears* (on seedlings):	G	S	G	S	G	S	G	S
Esperens Herre	10	8	10	9	10	5	10	0
% surviving	80		90		50		0	
(4) *Cherries* (on seedlings):								
Annonay	7	2	7	2	7	0	7	0
% surviving	29		29		0		0	

and one of sweet cherries (Annonay). The doses were 1,000, 2,500, 5,000, and 10,000 *r*-units. After treatment the scions were grafted as shown in Table 3, where the viability is also to be seen. As to apples, a higher percentage of survivors was obtained when the scions were double-worked on young nursery trees of known varieties than when E.M.IV was used as stock. Judging from the lethality observed, however, it was quite clear that about 5,000 *r* was the most suitable dose for apples and pears. For cherries the threshold value seemed to be somewhat lower, presumably about 2,500 *r*. No special effects on stems or leaves were noticed on the surviving material. The treatment of the scions was applied rather late in the season, the buds of the cherries already being in their first growth.

1947. — The treatment comprised scions of four apples (the diploids James Grieve and Cox's Orange, the tetraploids 37/60 and 38/1 — both progenies of the triploid variety Belle de Boskoop; NILSSON-EHLE, 1938; BERGSTRÖM, 1938), of three pears (the diploids Esperens Herre and Williams = Bartlett, the triploid Moltke), and of one sweet cherry (Frogmore). Apples and pears were treated only with 5,000 *r*-units, cherries with 1,000 and 2,500 *r*. The apple scions were grafted on nursery trees of different varieties, the pear scions on an old Sockerpäron, called »Tree K» (»Sugar Pear» = Kleine Gelbe Frühbirne). The cherry scions were, unfortunately, badly damaged by frost during the preceding severe winter 1946/47. They all died and are excluded from Table 4.

TABLE 4. *Number of grafted (G), and surviving (S) scions from the trial of 1947.*

	5,000 *r*	
(1) *Apples* (on young trees):	G	S
James Grieve	16	10
Cox's Orange	17	8
37/60	12	4
38/1	14	5
Σ	59	27
% surviving	46	
(2) *Pears* (on »Tree K»):		
Esperens Herre	14	7
Williams	14	4
Moltke	16	11
Σ	44	22
% surviving	50	

Some of the apple and pear scions showed abnormal agglomerations of leaves, and in some cases the leaf-shape also deviated from the normal type. As a rule the leaves at the agglomerations were narrow, on other places of the scion some leaves with bifurcated midrib and surface appeared. On four of the Cox's Orange and one of the James Grieve scions twin or split buds appeared, which gave rise to single or repeated bifurcation of the shoots.

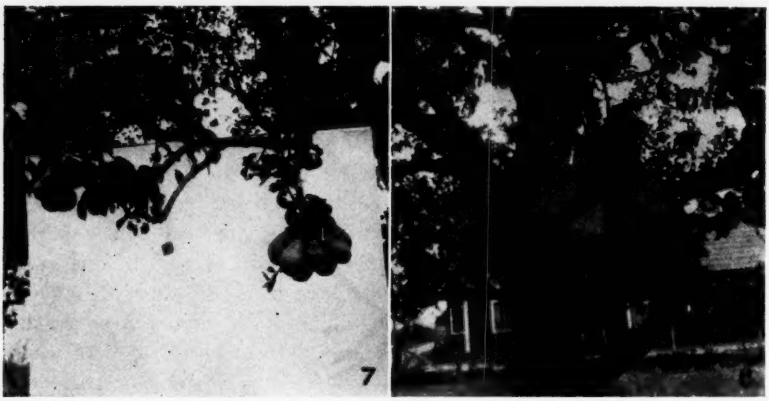

Fig. 7. The scions A (to the right) and B (to the left) of Williams 1947—5,000 *r* in 1948. — Fig. 8. Scion B of Williams 1947—5,000 *r* with 7 pointed pears in 1948. Note also the leaf agglomerations.

1948. — Three apple varieties (the diploid Cox's Orange, the triploid Gravenstein, and the tetraploid 37/60), two pears (the diploid Williams and the triploid Moltke), and two cherries (the diploid Frogmore and a spontaneously arisen tetraploid *Prunus Avium*; DANIELSSON, 1947) were treated. The dosage was 5,000 *r*-units for apples and pears and 2,500 *r* for cherries. The viability is evident from Table 5. Visible effects occurred on several of the surviving scions, namely on 13 Cox's Orange, 3 Gravenstein, 0 37/60, 33 Williams, 14 Moltke, 5 Frogmore, and 1 tetraploid *P. Avium*. The same bifurcations of shoots and leaves, deformations and agglomeration of leaves already described were noted. It was interesting to observe that the percentage of affected scions was higher in the diploid than in the triploid and tetraploid apple and pear varieties. As to cherries, the number of living tetraploid scions is too low (probably depending upon the late time of grafting) to permit

comparison. The luxuriant and coarse cell tissues of the tetraploid seemed especially sensitive at this point.

TABLE 5. *Number of grafted (G) and surviving (S) scions from the trial of 1948.*

	2,500 *r*		5,000 *r*	
(1) *Apples* (on nursery trees):	G	S	G	S
Cox's Orange	—	—	42	18
Gravenstein	—	—	38	27
37/60	—	—	45	29
Σ	—	—	125	74
% surviving		—		59
(2) *Pears* (on seedlings):				
Williams	—	—	50	46
Moltke	—	—	52	51
Σ	—	—	102	97
% surviving		—		95
(3) *Cherries* (on nursery trees):				
Frogmore	24	16	—	—
Tetraploid *P. Avium*	24	2	—	—
Σ	48	18	—	—
% surviving		(38)		—

TREATMENT EFFECTS IN THE FOLLOWING YEARS.

The experiments of the first years, 1944—46, have indicated which X-ray doses are lethal to scions of apples, pears, and sweet cherries. Only a few and insignificant habitual effects of the treatment were, however, observed in the scions in the first season. In the summer of 1948 there were three cases of possibly lasting effects noted in this material, all concerning fruit colour. At Alnarp one of the Åkerö 5,000 *r* scions from 1944 developed fruits with striped blush (normal blush light brick-red without stripes). A Gravenstein 1945—5,000 *r* scion, also at Alnarp, had fruits with a slightly stronger red blush than usual. A note was also made that the shape of the fruits of these two Alnarp scions slightly deviated from the normal types of the respective varieties. At Balsgård a Cox's Orange 1946—10,000 *r* scion also showed a somewhat deeper red fruit colour (9 fruits) than the apples of neighbouring untreated Cox's Orange trees. Similar spontaneous fruit colour sports are often described (*e. g.* striped Åkerö, red Gravenstein, and red Cox's Orange; see DAHL, 1943). Further observations on the fruit colour of the

three scions are necessary to make clear whether the deviations of 1948 are really permanent and unmistakable. It seems probable, but the evidence is in no way complete, that colour variations have really been induced by the X-ray treatment.

In 1947 and 1948 very pronounced primary X-ray effects on the development of the scions were observed in the new material.

During the summer of 1948 investigations on the 1947 material showed that many of the typical deformations still remained in the overwintering parts of the scions and also continued to appear in the new shoots. There were several cases of furcated leaves and other ab-

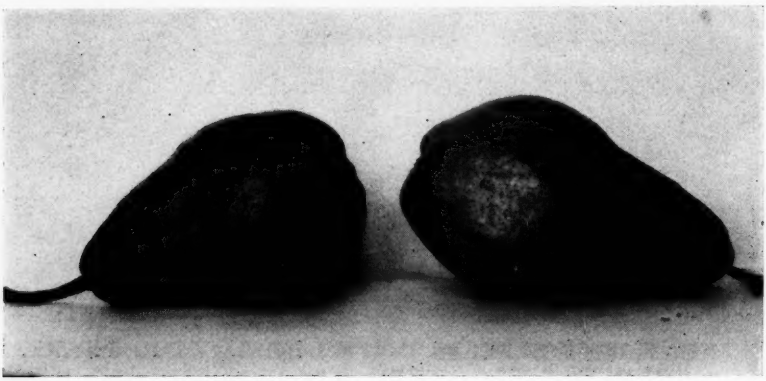

Fig. 9. Pear of Williams scion B (to the left) and scion A (to the right) in 1948.

normal leaf shapes in the apple and pear material. Formation of double buds on the new wood was also observed in some cases in Cox's Orange, giving rise to new bifurcated shoots.

Of the pear variety Williams two scions (here named A and B and both grafted on the southern low branches of »Tree K»), which were treated with 5,000 *r* in 1947, developed flowers and also fruits in 1948. Scion A had seven pears, all unusually well developed with weights from 235 to 305 grams, average: 274 grams. Scion B had four pears, weighing 175 to 260 grams, average: 236 grams. All these weights are remarkably great. The pears of each cluster were very uniform among themselves, but there was a clear difference in fruit shape between the two groups. On the scion A the pears had a pointed type. On the scion B the top of the pears was more flattened than that of A (Fig. 10). The broadest part of the fruit was nearer to the top in B than in A. The

mother tree of the scions, which is growing in the orchard of the estate of Råbelöf near Balsgård, has fruits of the type B. There were no untreated control scions of Williams borne by »Tree K», and it was consequently impossible to compare the size and weight of the »X-ray

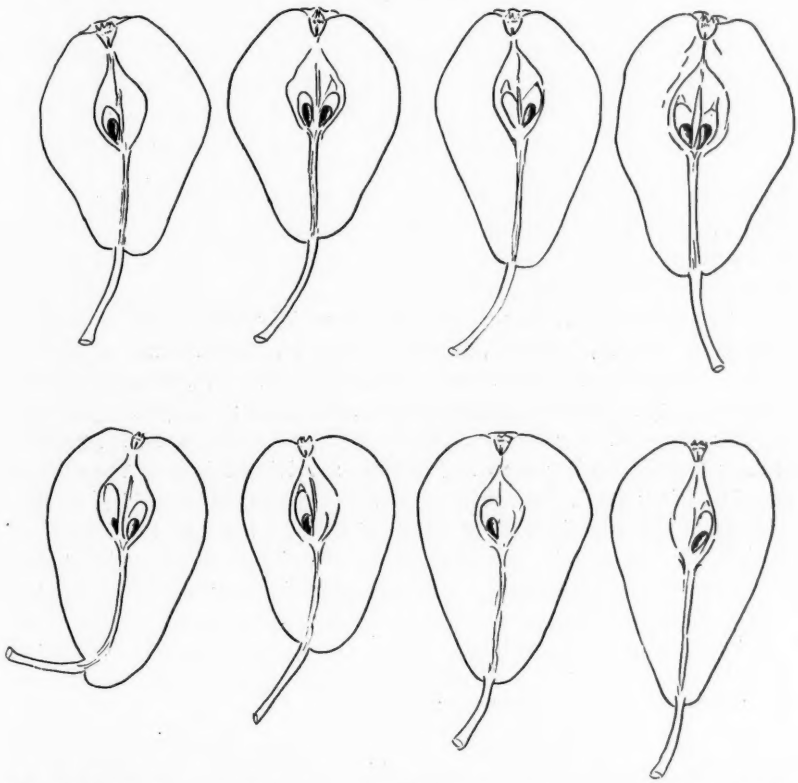

Fig. 10. Fruit shape of »X-ray pears». Williams scion A (upper row) with pointed top and low »equator». Scion B (lower row) with flat top and high »equator».

pears» in an exact manner with those of normal Williams in 1948. Another interesting observation was the date of ripening. All other Williams pears in the neighbourhood of Balsgård ripened during the first week of September. The pears of scion A, however, did not ripen until September 21st, and those of scion B ripened as late as September 28th. Of course, environmental conditions may at least partially

be responsible for the difference in ripening time between ordinary Williams on the one side and the two scions A and B on the other. The difference between A and B was, however, quite indisputable.

DISCUSSION.

In agricultural plants induced mutations have already proved their scientific and practical value (GUSTAFSSON, 1947). In barley, *e. g.*, genes influencing time of ripening, straw strength, grain yield and also quality properties mutate frequently after X-ray treatment. The authors have tried to perform similar experiments with fruit trees. In this connection it may be worth mentioning that as early as in 1930 STADLER characterized the possibilities of practical results by induced mutations as more promising in the breeding of fruit trees than in that of sexually reproduced agricultural plants.

The experiments described above are mainly of a preparatory character. They have, however, shown what the efficient doses of X-rays are for apples, pears, and sweet cherries and revealed some differences in sensibility between varieties of these fruits. The most typical primary effects are the bifurcations of shoots and leaf midribs. In the experiments of 1948 no less than 72 % of the surviving scions of the diploid apple variety Cox's Orange showed these greater effects, 11 % of the scions of the triploid variety Gravenstein, and none of the tetraploid 37/60. Smaller effects (mainly leaf deformations) appeared in all the three varieties. In pears the corresponding figures were 72 % for the diploid variety Williams and 27 % for the triploid variety Moltke. The variety number is insufficient to allow any general conclusion, but it is interesting to note the correlation between chromosome number and resistibility to treatment. The influence of polyploidy is known from other genera, *e. g. Triticum* and *Avena*, where spontaneous and induced mutations are more seldom met with in the higher polyploid stages than in the lower ones (STADLER, 1930; FRÖIER, GELIN and GUSTAFSSON, 1941).

Most of the abnormalities described here sometimes occur in nature and are familiar to most fruit growers. The frequency of their appearance in nature seems to depend upon different environmental factors and also on the variety. In apples bifurcated shoots are presumably more common in Cox's Orange than in most other varieties. In the nursery at Balsgård bifurcated leaves were found in Williams (*cf.* Fig. 5) but not in Moltke. The X-ray treatment consequently seems to

increase already existing abnormal tendencies. Further observations may reveal whether these types of effect are real vegetative mutations or (most likely) derive from local damages at the points of vegetation.

The induction of real vegetative mutations in fruit trees by means of X-rays is still insufficiently investigated, but the observations made in 1948 — especially on the Williams pears — would seem to be rather promising. For the immediate future interest will be directed especially towards the investigation of mutation type, frequency, and utility. Similar experiments are planned with other mutagenic agents, *e. g.* neutrons, radioactive substances, and mustard gas.

If vegetative mutations can be induced on a larger scale in this way, it means that single practically important characters or complexes of characters can be changed, *e. g.* colour, size and shape of fruits, time of ripening, and — why not — also essential physiological characters like winter hardiness, disease resistance, vitamin content, and palatability. In comparison with the common crossing method of effecting variation the mutation method means that the main mass of genes remains unchanged. In fruit trees which are generally extremely heterozygous, crossing causes a very wide and elusive variation, often also including a severe breakdown of the gene balance and the adaptation of the parental varieties. Possibly, the induced mutations will often give rise to chimaeras, which may hamper the breeding work and cause difficulties at the vegetative propagation. As far as can be judged at present, however, the mutation method seems to offer many new theoretical and practical views and possibilities.

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COLCHICINE, X-RAYS AND THE MUTATION PROCESS

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IN their paper of 1948 D'AMATO and GUSTAFSSON examined the combined effects on the mutation process of various chemical substances and X-rays. They claimed that a treatment with colchicine solutions, previous to the irradiation, caused the origin of rare mutations and mutation types, which do not readily appear in X-ray experiments involving dry and dormant seeds. Owing to the reach of this finding we have found it necessary to re-examine the problem and gather new data.

The seed material of this as well as the previous investigation belonged to a special strain, 01518, out of Ymer barley. Twenty-four hours before irradiation seeds were set to germinate in five different colchicine solutions of 0,1, 0,05, 0,01, 0,005 and 0,001 % strength. The four first-mentioned concentrations cause a complete c-mitosis in barley roots. The lowest concentration, on the contrary, is not able to produce this effect (LEVAN, 1946). The X-ray doses applied consisted of 5,000, 10,000 and 15,000 r-units. They were immediately given after the end of the pretreatment. The irradiation was performed at the Radiophysics Institute, Stockholm, under the supervision of Dr. A. FORSSBERG. Dormant and water-treated seeds were given similar X-ray doses as controls. The resulting X_1 plants were grown and harvested at Svalöf under the standard conditions of the Swedish Seed Association. Two or three spikes per plant were sown indoors in sand, in each series approximately 200—300 spikes from up to 100 X_1 plants. The chlorophyll mutations were analysed with regard to colour, phenotypical behaviour, type of segregation and the fertilities of the corresponding X_1 plants and spikes.

The amount of material, as well as the number and types of mutations are shown in Table 1.

There is no linear relationship of mutation rate and dose in the experiments reported here. This agrees with previous results obtained by FREISLEBEN and LEIN, 1943, as well as GUSTAFSSON, 1947. The mutation rate, calculated per spike progeny and r-unit, drops from $5{,}4 \cdot 10^{-6}$ at 5,000 r to $1{,}8 \cdot 10^{-6}$ at 15,000 r for the dry seed series and

TABLE 1. *Number and type of mutations in irradiated colchicine series.*

Seed series	No. of X_1 plants	No. of X_1-spikes	Type of mutation					Σ	Mutation rate per spike progeny and r-unit
			Albina	Viridis	Xantha	Albo-viridis	Others		
C_1: 0,1 % colch.									
5,000 r	83	235	5	3	—	1	—	9	$7{,}6 \cdot 10^{-6}$
10,000 r	98	275	6	6	2	1	1	16	5,8
15,000 r	81	238	6	3	1	2	—	12	3,4
C_2: 0,05 % colch.									
5,000 r	87	246	1	—	1	—	—	2	$1{,}6 \cdot 10^{-6}$
10,000 r	96	264	2	1	—	3	—	6	2,3
15,000 r	98	267	2	—	1	—	—	3	0,7
C_3: 0,01 % colch.									
5,000 r	87	248	3	1	2	1	1	8	$6{,}5 \cdot 10^{-6}$
10,000 r	65	189	—	1	—	1	—	2	1,1
15,000 r	93	269	4	3	—	—	2	9	2,2
C_4: 0,005 % colch.									
5,000 r	74	215	1	1	—	1	1	4	$3{,}7 \cdot 10^{-6}$
10,000 r	78	227	2	1	—	—	—	3	1,3
15,000 r	99	279	8	2	1	1	3	15	3,6
C_5: 0,001 % colch.									
5,000 r	89	251	4	1	—	—	1	6	$4{,}8 \cdot 10^{-6}$
10,000 r	92	257	3	4	2	1	1	11	4,3
15,000 r	93	267	7	3	1	—	—	11	2,7
H_2O									
5,000 r	49	139	3	2	—	—	—	5	$7{,}2 \cdot 10^{-6}$
10,000 r	30	90	—	1	—	—	1	2	2,2
15,000 r	97	285	5	2	—	2	1	10	2,3
Dry seeds									
5,000 r	175	448	7	4	—	1	—	12	$5{,}4 \cdot 10^{-6}$
10,000 r	191	453	8	6	—	—	2	16	3,5
15,000 r	194	505	6	5	1	2	—	14	1,8
Σ	2049	5647	83	50	12	17	14	176	$3{,}4 \cdot 10^{-6}$

from $7{,}2 \cdot 10^{-6}$ to $2{,}3 \cdot 10^{-6}$ for the water-treated seed series. The average figures for the colchicine series at 5,000, 10,000 and 15,000 r-units respectively are 4,8, 3,1 and $2{,}5 \cdot 10^{-6}$.

In accordance with previous findings the colchicine treatments did not specially augment the mutation rates, possibly with the exception of the highest concentration. With the 1948 irradiation of 7,500 r-units

TABLE 2. *Rates of different mutation types, calculated per 1,000 spike progenies.*

Seed series	Common mutants		Rare mutants		Very rare mutants	Sum of mutants per 1,000 spike progenies
	Albina	Viridis	Xantha	Alboviridis		
Dry seeds 1948	13,1	13,1	1,6	1,6	0,0	29,5
C_1—C_3 1948	13,0	9,0	5,1	3,0	6,5	36,8
Proportion 1948	*0,99*	*0,69*	*3,19*	*1,88*	(∞)	—
Dry seeds 1949	14,9	10,7	0,7	2,1	1,4	29,9
C_1—C_3 1949	13,5	7,5	2,7	3,7	2,7	30,1
Proportion 1949	*0,91*	*0,70*	*3,81*	*1,75*	*1,91*	—
H_2O 1948	22,0	8,0	2,0	2,0	4,0	38,2
H_2O 1949	17,5	7,8	0,0	3,9	3,9	33,1
C_3 1949	18,0	10,3	3,9	1,3	2,6	36,1

there was an average mutation rate of $4{,}9 \cdot 10^{-6}$ against $3{,}9 \cdot 10^{-6}$ in the dry seed series. The average figures of the 1949 experiments are $3{,}4 \cdot 10^{-6}$ as against $3{,}6 \cdot 10^{-6}$, *i. e.* in neither case a certain difference.

With regard to the type of mutation the data of 1948 and 1949 agree remarkably well, as indicated by Table 2. In order to obtain exactly comparable figures we have referred the various mutation rates to a number of 1,000 spike progenies.

The data evidence that a treatment with colchicine previous to the irradiation decreases the number of viridis types, greatly increases the number of rare and very rare mutants but causes no change in the number of albinas. Water treatment apparently augments the number of very rare types, possibly also of albinas, and decreases the number of viridis types. The weakest colchicine solution, producing no c-mitosis, has an effect similar to that of water treatment alone.

Grouping the mutants into three categories, as done in the 1948 paper, we find the following conditions (see next page).

Contrasting the colchicine-treated and the dry seed material we find a χ^2 of 10,283, which for two degrees of freedom corresponds to a P value of less than 0,01. Other groupings of the material produce P values around 0,001. Consequently, the chief result of the 1948 paper, *i. e.* the possibility of an experimental control of the mutation process, is, we consider, definitely corroborated.

In several papers (GUSTAFSSON, 1940, 1946; D'AMATO and GUSTAFSSON, 1948) the claim has been made that xantha mutations do not generally appear in the offspring of normally fertile X_1 plants and spikes.

	Common mutants	Rare mutants	Very rare mutants	
5,000 r: C_1—C_4	15	6	2	= 23
7,500 r: C_1—C_4 (1948)	51	19	15	= 85
10,000 r: C_1—C_4	19	7	1	= 27
15,000 r: C_1—C_4	28	6	5	= 39
5,000 r: dry seeds	11	1	0	= 12
7,500 r: dry seeds (1948)..	16	2	0	= 18
10,000 r: dry seeds	14	0	2	= 16
15,000 r: dry seeds	11	3	0	= 14
Σ C_1—C_4	113	38	23	= 174
Σ Dry seeds	52	6	2	= 60

In order to arise they require for some reason or other such a profound X-ray action upon the genotypical constitution that a conspicuous plant and spike sterility will result. The present mutation material confirms this view. *No single xantha mutant* out of 23 appeared in the progenies of 90—100 % fertile X_1 plants in comparison with twenty-two albina mutants out of a total number of 140. The average fertilities of plants and spikes giving rise to different mutation types are as follows:

	Plant fertility	Spike fertility	No. of mutants
albina	73,4 %	76,0 %	140
alboviridis	72,7	77,7	26
xantha	70,7	68,0	23
viridis	68,7	69,3	84
very rare mutants	68,8	65,0	21

The material of xantha and very rare mutant types is not large enough to establish for certain that their ancestor plants possessed lower average fertilities than those of the albina group. But the difference between albina and viridis mutants in respect both to plant and spike fertility gives P values between 0,05 and 0,02, and the difference between albinas and xanthas with regard to spike fertility gives a P value of approximately 0,05. Hence there is hardly any doubt that our new material confirms the conclusions arrived at in previous investigations.

From the data presented here and in earlier papers we conclude that the course of the mutation process is considerably altered by the application of colchicine previous to the irradiation,

that xantha and alboviridis types, as well as very rare mutants, increase in number as a result of such a pretreatment,

that viridis mutants become rarer; and, finally,

that the different groups of mutation, at least with regard to albina, xantha and viridis types, show a conspicuous connection with the sterility conditions of the ancestor plants.

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APOMICTIC AND SEXUAL REPRODUCTION IN CALAMAGROSTIS PURPUREA

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SOME years ago the writer found that the apomictic species *Calamagrostis purpurea* (TRIN.) TRIN. only exceptionally produces any pollen. In 174 investigated clones from different parts of Sweden there was pollen present only in one. The clone in question, Gäll. 1—41, varied in pollen formation from one year to another. Further, there was in this clone an evident difference in regard to the type of division in various panicles depending on their age (NYGREN, 1946, p. 182). Thus, the panicles first developed during the vegetation period had P.M.C.'s with meiotic divisions, which principally followed the semiheterotypical scheme of ROSENBERG (1926—27), while younger ones had P.M.C.'s with mitotic chromosomes. The difference in division type between young and old panicles is very striking and is valid for E.M.C.'s as well. Thus, the first panicles have E.M.C.'s with meiosis, whereas later ones show diplospory with mitotic division. In 1943 the clone mentioned only developed two panicles, from which 6666 P.M.C.'s were examined. All these cells had chromosomes of meiotic type. When in 1944 a great number of panicles could be investigated, P.M.C.'s from five groups of different age were studied. The oldest group held 2672 cells, 30,8 per cent of which were meiotic, the next group including 122 cells had 8,2 per cent cells with meiosis, the third one was made up of 1191 cells with 7,1 per cent meiotic P.M.C.'s, while the two youngest ones containing together 5401 cells all had mitotic divisions.

As one and the same plant could show such a great variation in one year with regard to the type of division, and as, besides this, the capability of producing pollen on the whole seemed to decrease with age, the writer decided to investigate the manner in which the 173 clones without pollen would behave in this respect. Forty clones from different parts of Sweden were selected (NYGREN, 1948 a, p. 127). Panicles from these were bagged, and seedlings raised. The chromosome numbers of the seedlings were determined, in all 673 plants having been counted. Every seedling had the chromosome number of its apomictic mother, *viz.* $2n = 56$. The descendants were planted out

in the field in such a way that 12 plants from each of 34 clones were put in rows of four plants, while of the remaining ones, 25, 39, 57, 16, 35, and 21 plants respectively, were placed in single rows. In 1946 all plants had developed panicles, and therefore their capability of producing pollen could be investigated. Several panicles of different age were studied on every plant. This test gave the result that 31 of the 673 plants were found to have pollen (NYGREN, 1948 a, p. 128). The 31 plants in question belonged to eight of the selected forty clones. The reason why 31 descendants of eight apomictic clones could form pollen in spite of the fact that their mother clones had lacked pollen during the six—eight years they had been cultivated presumably depends on the circumstance that the plants had not been able to produce sufficient quantities of the substances necessary to start meiosis owing to the increase in the number of panicles after the first year. The first panicles in a young plant are always formed after a vegetative resting period of one or two years, during which time the root system is developing well. There is reason to presume that the quantities of meiosis-starting substances are sufficient when the plant flowers for the first time but that they are not sufficient the second year to allow the development of the great number panicles formed. According to this interpretation, the diplospory of *Calamagrostis purpurea* is thus caused by a lack of one or more substances. The experiment also shows that in this respect there is a gradation in different clones and plants. This is best elucidated by a comparison of the different frequencies of plants with pollen in the eight clones (Table 1).

TABLE 1. *Plants with pollen in eight of forty clones of Calamagrostis purpurea.*

Mother clone	Number of seedlings raised from the mother clone	Plants with pollen	*n*
J. 3—41	12	1—2, 2—3	2
J. 5—38	12	1—3, 1—4	2
Dorotea 2—41	12	1—2, 1—3, 1—4, 2—1, 2—2, 2—4, 3—1, 3—2, 3—4	9
S. 2—38 A	12	1—1	1
Gittafjäll 1—41 A	12	1—2, 1—3, 1—4, 2—1, 2—3	5
Jormlien 2—41 B	12	1—1, 2—1, 2—4, 3—1, 3—4	5
Robertsfors 1—41 B	12	1—4, 3—1, 3—2	3
A	12	1—1, 1—4, 3—1	3

The first figure in the second right column means the row, the second figure the plant number in the row.

The 31 plants with pollen were dug up and planted in large pots in an isolated place far from all other *Calamagrostis* cultures. All panicles on every number were bagged to prevent pollen from being transferred from one plant to another. Some of the panicles bagged were as soon as possible isolated together with panicles from the amphimictic species *C. arundinacea, canescens, epigeios,* and *varia.* In those cases in which the number of panicles was sufficient reciprocal crosses as well were made with the amphimictic species as mother plants. It is evident that such an encroachment as a transplantation just in the height of the vegetation period of these one to one and a half metre tall grasses entails poor seed-setting. There was, however, no other chance in the same season either of making a selection in a large material during the first year the plants were developing panicles or of cultivating the selected plants under satisfactory control. In some cases seeds have been obtained after bagging. They were sown out, and

TABLE 2. *Offspring of bagged Calamagrostis purpurea with pollen.*

Mother clone	Chromosome number of mother clone	The number of the I_a^1 plant	Chromosome number of the I_a^1 plant	Chromosome numbers of the I_a^2 plants
J. 3—41	$2n = 56$	1—2	$2n = 56$	$2n = 76, 80, 84$
Dorotea 2—41	$2n = 56$	1—3	$2n = 56$	$2n = 56$
		1—4	$2n = 56$	$2n = 56, 56, 56$
		2—2	$2n = 56$	$2n = 56, 56, 56$
		2—3	$2n = 56$	$2n = 56, 58, 70$
		»	$2n = 56$	$2n = 56, 56$
		»	$2n = 56$	$2n = 68, 69, 70, 70$
		2—4	$2n = 56$	$2n = 56, 56, 56$
		3—2	$2n = 56$	$2n = 63, 64, 68, 68$
S. 2—38 A	$2n = 56$	1—1	$2n = 56$	$2n = 63, 63, 66$
Gittafjäll 1—41 A ..	$2n = 56$	1—4	$2n = 56$	$2n = 65$
Jormlien 2—41 B ..	$2n = 56$	1—1	$2n = 56$	$2n = 69, 70, 70$
		2—4	$2n = 56$	$2n = 67, 70$
		»	$2n = 56$	$2n = 70, 71, 71$
		3—1	$2n = 56$	$2n = 56, 56, 56, 56$
		3—4	$2n = 56$	$2n = 56, 56, 56, 56, 56$
Robertsfors 1—41 B	$2n = 56$	3—1	$2n = 56$	$2n = 67$
A	$2n = 56$	1—1	$2n = 56$	$2n = 66, 66, 67$
		4—1	$2n = 56$	$2n = 66, 70$
		»	$2n = 56$	One plant, not determined

Descendants of bagged apomictic clones are denoted as I_a^1 plants. The next generations of these I_a^1 plants, raised after bagging as well, are designated I_a^2, and so on.

the descendants (I_a^2) were determined as to their chromosome numbers. The figures are found in Table 2.

It appears from Table 2 that various sister plants originating from *the same* mother clone behave differently as well as that divergent panicles on *the same plant* have given diverse results. This is clear from the figures relating to the clone Dorotea 2—41. In the I_a^2 the I_a^1 plants 1—3, 1—4, 2—2, and 2—4 have all given offspring with the chromosome number $2n = 56$ whereas the I_a^1 plant 3—2 has produced the series $2n = 63$, 64, and 68, while the I_a^1 No. 2—3 from one panicle has given rise to plants with $2n = 56$ only but two others to descendants with the numbers $2n = 56$, 58, 70, and $2n = 68$, 69, 70, respectively. The same condition is found in the offspring of the clone Jormlien 2—41 B. Here the I_a^1 plants 3—1 and 3—4 have given I_a^2 plants with $2n = 56$ only, while the I_a^1 numbers 1—1 and 2—4 produced higher chromosome numbers in the I_a^2. Thus it is evident that I_a^2 plants with the same chromosome numbers as the I_a^1 plants, *i. e.* $2n = 56$, originate by a diplosporous process. Plants with higher numbers have arisen in two different ways. Firstly, the egg-cell in a diplosporous E.S. has been pollinated with pollen from the plant itself. This has obviously happened in the clone J. 3—41, where the chromosome number $2n = 84$ is to be found in the I_a^2. Secondly, the egg-cell in sexual E.S.'s has functioned and been self-pollinated.

The present writer has earlier made it clear that the division in the E.M.C. of *Calamagrostis purpurea* may be meiotic (NYGREN, 1946, p. 171) and that this division principally follows the *Hieracium boreale* scheme of ROSENBERG (1917, p. 194). As a rule the number of bivalents in the E.M.C.'s of *C. purpurea* is high, and thus we have reason to expect most egg-cells in sexual E.S.'s to possess chromosome numbers near the number $n = 28$. In earlier crossing experiments with amphimictic species, above all *C. epigeios*, it has been proved to full evidence that male gametes with other numbers than even multiples of seven hardly function at all. The female gametes, however, may have very varying numbers without any effect on their capacity to function. For the bagged I_a^1 plants mentioned this means that the chromosome numbers of their functional egg-cells have been $n = 33$—43, 48, and 52, while the male gametes in all cases have had the number $n = 28$. This interpretation agrees with the data obtained from crosses between different chromosome races of *C. epigeios*. For that reason it must be considered as proven that I_a^1 plants — originated diplosporously from obligate apomictic clones — are capable of producing sexual male as

well as female gametes in spite of the fact that the original mother clones have never shown such an ability. Thus the mother clones in I_a^1 are revealed as being latently facultative apomicts, notwithstanding the fact that according to their cytology, embryology, and their macroscopically uniform offspring the original clone plants must be considered obligate apomicts. Moreover, it is of interest that the other I_a^1 plants in the investigation did not produce any pollen. It is not unlikely, however, that also these plants could develop sexual E.S.'s. Of the 174 clones in the investigation, 119 have been studied with regard to the type of division in the E.M.C. Not less than 17,6 per cent of these plants (*i. e.* 21 in number) had meiosis (NYGREN, 1946, p. 171). The sexual mechanism of reproduction in *C. purpurea* has thus been much more degenerated on the male than on the female side by the change to diplospory.

As was earlier mentioned, crosses were carried out with the 31 plants in question. Some of these crosses have been reciprocally made. In view of the limited number of panicles available for these experiments, however, *C. purpurea* was used as mother plant in most cases. The species *C. canescens*, *epigeios*, *arundinacea*, and *varia* functioned as father plants. The results are submitted in Table 3.

The crossing results confirm the figures found after bagging. In some cases, consequently, plants with the chromosome number of the mother clone only have arisen in the progeny, while in other cases the chromosome numbers in the offspring deviate from that of the mother. In the last-mentioned case we have three alternatives. Either pollen from the amphimictic species has functioned, the descendants thus being hybrids, or pollen from the plant itself has pollinated the egg-cell, or else the pollen of the plant itself only functions in the presence of pollen from another species in the genus, which stimulates it to develop. In the last-mentioned case hybrids as well as plants originated after self-fertilization occur.

In three cases offspring have been obtained from the same I_a^1 plant after bagging as well as after crossing. Dorotea 2—41, I_a^1 plant 1—4 after bagging gave three plants with the maternal chromosome number, $2n = 56$. After crossing with *C. arundinacea* the plant in question in one case gave birth to twelve descendants with the chromosome number $2n = 56$, in another case to ten plants with the maternal number and to two with numbers round about $2n = 70$. It is probable that the two descendants with high numbers are hybrids, as the I_a^1 plant seems unable to produce any reduced gametes on the female side. The I_a^1

TABLE 3. *Result of crosses between C. purpurea and different amphimictic species.*

Mother clone	I_a^1 plant	Chromosome number of I_a^1 plant	Father species	Chromosome number of father sp.	Chromosome numbers of descendants
J 3—41	2—1	$2n = 56$	*C. ep.* 4408	$2n = 56$	$2n = \overline{+}\ 58$
J 5—38	1—4	$2n = 56$	*C. can.* Källsta 1—42 A	$2n = 28$	$2n = 56, 56, 56, 56, 56, 56, 56$
Dorotea 2—41	1—4	$2n = 56$	*C. a.* Åsljunga 2—42 B	$2n = 28$	$2n = 56$ (12 plants)
	»	$2n = 56$	*C. a.* Åsljunga 2—42 B	$2n = 28$	$2n = 56$ (10 plants), 70, 70
S 2—38 A	1—1	$2n = 56$	*C. can.* Källsta 1—42 A	$2n = 28$	$2n = 54, 56, 57, 60, 62, 62, 63$
Gittafjäll 1—41 A ..	2—1	$2n = 56$	*C. a.* Innsbr. 6—42 A	$2n = 28$	$2n = 66, 66$
	2—3	$2n = 56$	*C. can.* Källsta 1—42 A	$2n = 28$	$2n = 64$
Jormlien 2—41 B ..	1—1	$2n = 56$	*C. can.* Eskilst. 3—41 A	$2n = 28$	$2n = 70, 72$
	»	$2n = 56$	*C. can.* Eskilst. 3—41 A	$2n = 28$	$2n = 77$
Robertsfors 1—41 A	1—4	$2n = 56$	*C. ep.* 4407	$2n = 56$	$2n = 56$
	»	$2n = 56$	*C. can.* Eskilst. 3—41 A	$2n = 28$	$2n = 49, 70, 70, 70$
	»	$2n = 56$	*C. can.* Eskilst. 3—41 A	$2n = 28$	$2n = 67, 70, 70, 70, 70, 70$
	»	$2n = 56$	*C. can.* Eskilst. 3—41 A	$2n = 28$	$2n = 67, 67, 67, 70$ (10 plants), 72.

The heading »Mother clone» has reference to plants from nature, while »I_a^1 plant» denotes descendants of the mother clone raised after bagging. The crosses were effected on these I_a^1 plants. In the column headed »Father species» *C. a.* = *Calamagrostis arundinacea*, *C. can.* = *C. canescens*, and *C. ep.* = *C. epigeios*. Below the species name the field number is given.

plant 1—1 of the clone S 2—38 A behaves in the same way in crossing as in bagging experiments. This also applies to the I_a^1 plant 1—1 of Jormlien 2—41 B. The greatest difference between bagged and crossed

panicles is shown by the I_a^1 plant 1—4 of the clone Robertsfors 1—41 A. This plant had a large number of panicles in the summer of 1946, and therefore a great number of isolations and crosses could be made. Four of the latter were successful, but all isolations failed (the number of bagged panicles exceeded ten). In this case it seems as though foreign pollen must be present to make seed-setting possible. It is probable that most descendants in crosses with this *C. purpurea* plant as mother are hybrids, as no fewer than 18 of them (or 75 per cent) have the chromosome number $2n = 70$, *i. e.* $56 + 14$. In one case a reduced female gamete seems to have been fertilized by pollen from the amphimictic species, giving rise to a plant with the number $2n = 49$. The fact that in four cases descendants have been obtained with the number $2n = 67$ probably depends on pollen of the plant itself having functioned together with reduced female gametes.

In 1946 the present writer found (pp. 229—230) that in *Calamagrostis* the plant's own pollen may be stimulated to function in obligate allogamous species in the presence of pollen from other species in the same genus. This phenomenon was, however, already observed by FAGERLIND (1937, p. 372) in the genus *Galium*. When results obtained in the U. S. A. during the last war became available in Sweden, it was found that the same observation had been made by CLAUSEN, KECK, and HIESEY in the *Madiinae* group of Compositae (1945, p. 9). The figures obtained for *Calamagrostis purpurea* make it probable that different biotypes in the same species may be stimulated in different degree. About the same figures as those obtained in the clone Robertsfors 1—41 A have also been found for Gäll. 1—41, *i. e.* the only clone with pollen found in nature. This clone has been bagged several consecutive years, but the results have always been the same. Either no germinable seeds have been produced, or the seeds obtained have given uniform offspring with the maternal chromosome number, $2n = 56$. In these cases diplosporous E.S.'s only have functioned parthenogenetically. After bagging the clone together with various amphimictic species in the genus the results have been diverse, however. In these cases germinable seeds have been obtained from practically all panicles bagged together, and the descendants raised have had the chromosome number $2n = 70$ or higher numbers (NYGREN, 1946, p. 232). Some new figures from similar crosses have confirmed the earlier results, as is clear from Table 4.

The figures from the crosses mentioned in Table 4 verify earlier results (NYGREN, 1946, p. 232, and 1948 a, pp. 121—123), but in addi-

TABLE 4. *Result of crosses between the Calamagrostis purpurea clone Gäll. 1—41 and different amphimictic species.*

Mother clone	Chromosome number of mother clone	Father species	Chromosome number of father species	Chromosome numbers of descendants
Gäll. 1—41	$2n = 56$	*C. epigeios* 4407	$2n = 56$	$2n = 67, 68, 68, 69$
»	$2n = 56$	*C. epigeios* 4408	$2n = 56$	$2n = 70$
»	$2n = 56$	*C. canescens* Eskilst. 3—41 A	$2n = 28$ (1)	$2n = 71, 71, 71, 72, 72$
»	$2n = 56$	*C. canescens* Eskilst. 3—41 A	$2n = 28$ (2)	$2n = 72, 72, 72$
»	$2n = 56$	*C. canescens* Eskilst. 3—41 A	$2n = 28$ (3)	$2n = 28, 28, 70, 71, 72, 72, 72, 72, 72, 72$
»	$2n = 56$	*C. canescens* Eskilst. 3—41 A	$2n = 28$ (4)	$2n = 72, 72, 72, 72$
»	$2n = 56$	*C. canescens* Eskilst. 3—41 A	$2n = 28$ (5)	$2n = 43, 71, 72, 72, 84$
»	$2n = 56$	*C. canescens* Eskilst. 3—41 A	$2n = 28$ (6)	$2n = 45$
»	$2n = 56$	*C. canescens* Frötuna 1—41	$2n = 28$	$2n = 56$ (32 plants determined)
»	$2n = 56$	*C. canescens* Trolleholm 1—41	$2n = 28$	$2n = 65$

In the column »Father species» the field number is given below the name of the species. The figures within brackets mean different panicles of the mother clone Gäll. 1—41.

tion to this something new has arisen. In two different crosses with *C. canescens*, Eskilstuna 3—41 A, two descendants in all have been raised, the chromosome numbers of which indicate that a reduced female gamete has functioned. On account of the low chromosome numbers of the two plants there is reason to suspect them to be hybrids between *C. canescens* and *C. purpurea*; the functioning male gametes thus having had the chromosome number $n = 14$, while the numbers of the egg-cells may have been $n = 29$ and $n = 31$, respectively. In two cases in the same cross (with *C. canescens*, Eskilstuna 3—41 A) plants with the chromosome number $2n = 28$ have arisen. It is possible that they are haploid *C. purpurea* plants, as the clone Gäll. 1—41 has earlier given rise to haploids (NYGREN, 1948 a, pp. 123—125). This cannot, however, be definitely established before panicles have developed. The large number of descendants with chromosome numbers at and about $2n = 70$ is easily interpreted in such a way that in these cases male

gametes from the amphimictic species have functioned with unreduced female gametes from *C. purpurea*. On the other hand, the number $2n = 65$ in one offspring indicates that in this case a reduced egg-cell has been self-pollinated. The results of the first two crosses with *C. epigeios* as father are explicable in like manner, though here it is possible that reduced female gametes have been fertilized with pollen from *C. epigeios* having the chromosome number $n = 28$. If this is true, the egg-cells would have had the numbers $n = 39$—42, a very probable eventuality.

DISCUSSION.

The species *Calamagrostis purpurea* (TRIN.) TRIN. constitutes the Northwestern-European part of an agamic complex (BABCOCK and STEBBINS, 1938, pp. 57—58) which is split into three form circles. East of *C. purpurea* the distribution area of *C. Langsdorffii* TRIN. begins, which later on is interrupted by *C. canadensis* (MICHX.) BEAUV. In the intervening zones, however, the three form circles are woven together and all kinds of transitional forms appear. The amphimictic species in the agamic complex are *C. canescens* (WEBER) ROTH and *C. villosa* (CHAIX.) GMEL. *C. canescens* is closely connected to *C. purpurea*, and *C. villosa* to *C. Langsdorffii*. *C. canadensis* is related to *C. Langsdorffii* by its variety *scabra* HITCHC. (HITCHCOCK, 1935, p. 315), and is thus considered to be most closely allied to the amphimictic *C. villosa*. The present writer has synthesized *C. purpurea* by crossing the two amphimicts *C. epigeios* ($2n = 42$, mother) and *C. canescens* ($2n = 28$, father). In three crosses seven hybrids were obtained among many hundred non-hybrid plants. All synthesized plants had the chromosome number $2n = 56$ (NYGREN, 1946, p. 225). A closer examination revealed the fact that the hybrids were devoid of pollen and that the E.M.C.'s developed diplosporously (NYGREN, 1948 a, p. 117). The seed-setting in the hybrid plants was very poor, a few solitary germinable seeds only were developed in the bagged panicles. In this respect synthesized *C. purpurea* behaved just like some of the I_a^1 plants in this investigation.

The most important difference between *C. canescens* and its hybrids with various amphimictic species on one side and *C. purpurea* on the other side is the type of reproduction. *C. canescens* is namely an amphimictic species, while *C. purpurea* is diplosporous. The most fundamental thing about the diplospory in *Calamagrostis* — as well as in most other diplosporous plant genera — is partly the absence of meiosis on the

female side — the division in the E.M.C. is mitotic — and partly the formation of E.S.'s without any degeneration of macrospores. Such forms as have diplospory caused by formation of restitution nuclei in the E.M.C., as is the case in *Ixeris dentata* (OKABE, 1932), are to be considered more primitive than those with obligate mitotic division in the E.M.C.

In 1946 the writer found (p. 241) that the mitotic type of division in the E.M.C. could occur in hybrids between amphimictic species. In a cross between *C. arundinacea* ($2n = 28$) and *C. epigeios* ($2n = 56$), the latter as mother plant, a hybrid was raised with the chromosome number $2n = 42$. This hybrid had some capacity to form mitotic divisions in the E.M.C. (about 0,16 per cent). From the hybrid some F_2 plants were raised that had varying chromosome numbers. In seven of these descendants with numbers between $2n = 38$ and $2n = 72$ the mitotical division was present in the E.M.C. (NYGREN, 1948 b, p. 406). In the F_1 hybrid meiosis on the male side was much disturbed; large plasmodia occurred in the loculi. Such plasmodia are typical of the apomictic *Calamagrostis* species *chalybaea* and *purpurea*. In the F_2 of the hybrid mentioned plasmodia were formed in not less than about 70 per cent of the investigated plants (*i. e.* in 25 of 36 in number). The conformity in disturbances between apomictic *Calamagrostis* species and the hybrid plant in question are obvious, but does not mean that *C. purpurea* must be interpreted as a species hybrid. It has certainly been synthesized by crossing two different amphimicts, but the variability in the *C. purpurea* complex is so manifold that there are probably more chances to obtain the same result. The fundamental condition is to bring together such genes in the F_1 plants as cause a disharmonious development of the generative phase without disturbing the vegetative one. This may, of course, appear as a result of intraspecific as well as of interspecific crosses. Thus it is not likely that apomicts as to their origin are species hybrids in the sense of ERNST (1918).

We have the right to presume that the generative as well as the vegetative phases in a plant species are regulated by substances which are brought in contact with all the different parts of the plant. Growth substances have long been known, and we have knowledge of the presence of some substances that are indispensable for the formation of flowers (HARDER, 1948). Thanks to an investigation by LEOPOLD and THIMANN (1948, p. 664) it is now evident that in low concentrations auxin increases the number of flower primordia in barley, while in higher concentrations it decreases the number of primordia in the same

plant. A similar effect is exercised by auxin on the formation of flowers in pineapple (CLARK and KERNS, 1942, pp. 536—537). Further, PESONEN has shown (1946) that meiosis in the ovaries of mice starts on supply of a hormon, prolan A, from the anterior lobe of the hypophysis. These facts point in the same direction: growth and reproduction in plants and animals are dependent on the presence of specific substances. If there is a decrease or an increase of these substances in relation to the quantities normal to the particular species, the result will be disturbances in one or in both phases of development. HARDER's investigations in *Kalanchoe Blossfeldiana* (1948, p. 119) have shown that the development of flower-initiating substances in that species is dependent on the length of day, *i. e.* on an outer factor. All transitions between full anthesis and a purely vegetative stage could be obtained in *Kalanchoe* by varying the exposure to light. This indicates that the substances indispensable to the vegetative development have gained superiority over those initiating the formation of flowers.

In *Calamagrostis purpurea* quite a different behaviour in the type of division for panicles of different age has been demonstrated for clone Gäll. 1—41. The first panicles had meiosis, the later ones produced mitosis. Broadly viewed, this applies to P.M.C.'s as well as to E.M.C.'s. As mitosis is common to all tissues of the plant but meiosis is limited to those producing gametes, the results obtained from *C. purpurea* favour the belief that a decrease has arisen in meiosis-starting substances, with the result that the substances of the vegetative phase have taken over the starting of divisions in the generative tissues as well. Thus we obtain mitosis instead of meiosis in a predominant number of flowers in the panicle. The plant has, however, a predisposition to start meiosis, which has a chance to appear in later generations. This is the case only if seedlings are able to grow up. It is, however, typical of *C. purpurea* that this species principally propagates with stolons. Seedlings very seldom develop, though this sometimes happens when the species colonizes the inundation belts along lakes and rivers.

Summing up the data concerning *C. purpurea*, we learn that it originates from *C. canescens* and the hybrid derivatives of that species. The original hybrids first formed are very sterile and have — besides a disturbed meiosis on the male as well as on the female side — mitotic division followed by diplospory in the E.M.C. The male meiosis is ultimately broken down completely, which entails total incapability to produce pollen. This developmental cycle is applicable not only to different generations but also to the plant itself. The same individual thus

has the capacity of forming gametes by a sexual method during the very first year, but this capacity decreases later on and it finally disappears. The plant is transformed into an obligate apomictic stage. Descendants of the original clone may, however, unveil the real nature of the plant as a facultative apomict. The period during which the ability to reproduce sexually lasts is very short compared with the time during which the plant propagates agamospermically. This is the reason why most *C. purpurea* plants in nature have the chromosome number $2n = 56$. Other numbers (*viz.* $2n = 63$—91) are in Sweden principally found in some districts in the Northern and Middle parts of the country. This probably depends on the circumstance that in these areas the seedlings find it easier to survive by striking root in the inundation zones of lakes and rivers and from there to spread by vegetative methods. Thus, the polymorphy of the species is to a very high degree dependent on the condition of the surrounding ground. Under good germinating conditions a large number of facultative apomictic clones may be developed, which results in the formation of hybrids as well as in an increase of the polymorphy. When the external conditions for the seedlings to survive are poor, the areas are also poor in forms. This is the reason that in large parts of Sweden a solitary *C. purpurea* clone is the only dominating one. The circumstances mentioned mean that apomixis in *C. purpurea* is rather to be interpreted as a modification than as a dominant or recessive quality. The species thus constitutes an analogous case to *Deschampsia caespitosa* (L.) PB., in which vivipary has been produced by changing the length of daylight and the life rhythm of the plant as well (NYGREN, 1949).

A survey of the chromosome numbers in the 174 *C. purpurea* clones shows that 35 of them have higher numbers than $2n = 56$ (NYGREN, 1946, p. 202). These derivatives have originated, as was explained earlier in this paper, by the fertilization of a reduced or an unreduced female gamete from *C. purpurea* with this species' own pollen or with foreign pollen. In culture all these clones behave as obligate apomicts, however. Thus they give maternal offspring without deviations as to chromosome number. From the preceding remarks, however, it is clear that a test — to be valid — must be made more thoroughly by performing cytological and embryological investigations on the plants and their descendants.

From the investigations of MÜNTZING (1940) and ÅKERBERG (1939, 1942) in *Poa alpina* and *P. pratensis* as well as their hybrids we know that apomixis may be broken down by crossing. It is therefore to be

suspected that the hybrids between these two species would reproduce sexually in nature. This is not the case, however. The present writer has investigated a great number of plants from nature of viviparous *Poa herjedalica* H. SMITH, *i. e.* the hybrid between viviparous *Poa alpina* and *Poa pratensis*. All plants investigated produce aposporous E.S.'s — often in great numbers — in the nucellus, in spite of the fact that owing to their viviparous mode of propagation they have very small chances of agamospermous development. The investigations embrace hybrid plants with very varying chromosome numbers from different parts of the Scandinavian mountain ridge, and therefore these plants probably constitute various gamete combinations of the parents. The species would obviously reproduce in an aposporous way if vivipary could be removed. Sexual E.S.'s are sometimes formed, however, and thus variations in the chromosome numbers as well as in the morphology are to be expected in such a case. Apomixis in *Poa* can be broken down by hybridizing, but it may also be restored in later generations. CLAUSEN, KECK, and HIESEY write (1947, p. 103): »The possibility exists in *Poa* that when hybrids between apomictic species are formed which reproduce sexually, these may in later generations produce apomictic forms again.» Since this was written, new facts arguing more clearly in the same direction have come to light. Thus CLAUSEN writes (in a letter): »In all of our crossings both parents were apomictic. Each combination produced some apomictic and some sexual F_1's. Some of the sexuals become apomictic again in the F_2, producing constant F_3's.» CLAUSEN, KECK, and HIESEY comprehend their views in the following words: »From these seedlings, however, it is already evident that asexually reproducing forms may arise in the third generation from sexual hybrids that originated as offspring of partially asexual species. This makes possible the selection of new selfperpetuating strains from widely segregating hybrid populations and greatly increases the possibilities of finding new desirable strains» (1948, p. 105). These facts are in agreement with the interpretation given by GUSTAFSSON (1947, p. 253) regarding the origin of the polymorphy in the critical groups of *Poa alpina* and *P. pratensis*.

A comparison between *Calamagrostis purpurea* and *Poa* makes it probable that in both genera facultative sexuality plays a more important rôle at the formation of polymorphous form circles than autosegregation (GUSTAFSSON, 1942, p. 269). The presence of pseudogamy in the genus *Poa* is probably the cause that hybrids relatively often originate in nature, while in *Calamagrostis purpurea* the environment,

the chances for the seedlings to develop and other similar circumstances are the most important ones.

For practical plant-breeding the results obtained in both genera constitute a decided advance. It is now possible to obtain from apomicts offspring — hybrids or descendants — which behave as sexuals but in later generations give rise to new apomicts again. No doubt we have reached the goal that in 1947 loomed before CLAUSEN, KECK, and HIESEY when they wrote (p. 103): »It would indeed be of considerable practical and theoretical importance were we able to break the apomictic bond periodically, releasing the variability and then sealing it up again after a period of recombination.»

SUMMARY.

(1) The apomictic species *Calamagrostis purpurea* (TRIN.) TRIN. is devoid of pollen. Only one clone of 174 investigated ones formed pollen. Forty of the remaining 173 clones have been bagged, and in all 673 descendants (I_a^1 plants) have been raised from seeds of the isolated clones. All these plants had the maternal chromosome number, *viz.* $2n = 56$. Thirty-one of the 673 plants — belonging to eight clones — had pollen.

(2) The I_a^1 plants producing pollen have been bagged as well as crossed with various amphimictic species. Some bagged plants gave offspring with the maternal chromosome number only, while others produced descendants with deviating numbers. On the other hand, some plants from one panicle produced offspring with maternal numbers, but from other panicles developed descendants with fluctuating numbers. In those cases, where deviating numbers occurred after bagging, reduced as well as unreduced egg-cells have been fertilized by pollen from the plant itself. The figures from the crosses indicate that in some cases foreign pollen has stimulated the plant's own pollen to function. The obligate apomictic mother clones have revealed themselves to be facultative apomicts.

(3) An obvious decline of the sexual reproduction from one year to another has been proved for only the *C. purpurea* clone found with pollen from nature. This is supposed to depend on a lack of substances starting meiosis. In *C. purpurea* such substances are presumed to be formed in sufficient quantities during the very first year when the panicles are developed. Later on the effect is gradually diluted and those substances which regulate the vegetative phase of the plant be-

come dominant. As a consequence the divisions in the E.M.C.'s are transformed into mitosis. The occurrence of diplospory in *C. purpurea* might therefore be interpreted as being due to the lack of such substances.

(4) The results obtained in *Calamagrostis* and *Poa* corroborate the conclusions regarding the origin, breaking down, and recombination of apomixis given by CLAUSEN, KECK, and HIESEY. The breeding of apomicts should thus be worked out in that way that apomixis is broken down by suitable crosses. In later generations recombination of apomixis is possible; normally this occurs in F_3.

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STUDIES ON X-RAY MUTATIONS IN THE WHITE AND FORKED LOCI OF DROSOPHILA MELANOGASTER

II. A STUDY OF THE FORMATION OF GYNANDROMORPHS AND OTHER KINDS OF MOSAICS

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I. INTRODUCTION. MATERIAL.

IN a recent paper BONNIER and LÜNING (1949) presented data from X-ray studies on mutation rates in the white and forked loci of *Drosophila melanogaster* together with a statistical analysis of these data. As was mentioned there, this paper will consider in detail some of the special cases that arose in these experiments and also present a discussion of the results of the whole series of experiments. In addition, data from a new experimental series to be published in detail at a later date have been included in so far as they throw light on the problems at hand.

The material for the present paper consists, thus, of two different groups of data, *viz.* the material from the foregoing paper and new material. To distinguish between these, the first group will here be named the »old series» and the latter group the »new series». In the old series wild males were irradiated by X-rays of different doses and crossed to either white forked or to yellow white forked Beadex females. In the new series wild males of the same stock were irradiated by X-rays at 2908 *r* and crossed to yellow white singed females. (We are indebted to Miss CH. AUERBACH for the *y w sn* strain.) The animals which will be dealt with here belong to the F_1 progeny from these crosses. In the record numbers of the F_1's of the old series one of the following signs are included: I, III, V, VII, X, XI or K. The animals of the new series contain in their record numbers one of the signs A, B, C or D.

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II. DISCUSSION ON THE FORMATION OF GYNANDROMORPHS.

1. THE STAGE AT WHICH ELIMINATION OCCURS.

The hypothesis of MORGAN (1914; MORGAN and BRIDGES, 1919) that in the great majority of cases *Drosophila* gynandromorphs are due to elimination of one *X* chromosome at an early cleavage mitosis of a female zygote has been generally accepted. STURTEVANT (1929) found in *Drosophila simulans* a mutant, claret, of such a kind that, apart from other peculiarities, more than 3 percent of claret »females» were gynandromorphs produced by the elimination of a maternal *X* chromosome. From a study of the distribution of female and male parts on the body surface of 96 gynandromorphs he concluded that the elimination occurred chiefly, or perhaps exclusively, at early cleavage divisions and probably always before the segregation of the imaginal discs. In view of the fact that about 15 percent of the gynandromorphs were more than half-male he supposes that these are due to more than one elimination.

PATTERSON (1931) studied gynandromorphs produced after irradiation with X-rays and these studies were extended and described in more detail by PATTERSON and STONE (1938). These authors made a thorough description of 213 gynandromorphs of which 30 were spontaneous and the rest X-ray induced. By studying the distribution of male and female parts they found that the results could best be explained by the hypothesis that there is a high rate of elimination at the first cleavage division but that thereafter this rate is very much lower. They seemed, however, not to have supposed that the elimination always, or practically so, occurred at the first cleavage division, since they stressed that, if this were the case, the amount of male and female tissue should be nearly equal, whereas they found that the proportion between the parts of the two sexes (or more correctly the proportion of the imaginal discs from which male and female tissue developed) were 2 female to 1 male. But if the start of the X-ray induced elimination process could be postponed to a later stage in the embryogeny it would be somewhat difficult to understand how the eliminating forces are acting. It is therefore of interest to know if, in spite of the 2 : 1 proportion, it is necessary to suppose that not all eliminations occur — or more precisely are initiated — at the very outset of the embryonic development. With this purpose in mind we will here compare our

results concerning body-parts of mixed male and female origin with those of PATTERSON and STONE (1938).

Within the old series we found 172 gynandromorphs. During the earlier part of these experiments the nature of the gynandromorphs was only described by a few words in the records of our co-workers. Later we began to make descriptions on special cards. Detailed descriptions of each gynandromorphs were, however, not made until the beginning of the new series. In this series we have found altogether 145 gynandromorphs (all occurring from the cross of irradiated wild males to *y w sn* females), and of these, 140 have been described on special cards. (The five specimens which were not described were lost before descriptions could be made.) As a rule, one of us, LÜNING, made a first description of the etherized specimen immediately after its observation. It was then laid in alcohol for a few days and another, thorough description was made by BONNIER. The parts described included all bristles of the body (according to DIS worksheet No. 1 by C. B. BRIDGES), the eyes, ocelli, antennae, proboscis, palps, legs, wings, tergites, sternites, and external genitalia. Of these descriptions, those of the sternites are not always reliable, since the yellow body colour is often difficult to distinguish from the wild colour on the ventral side and since singed does not always show distinctly enough on that side. The distribution of male and female parts on the external genitalia may likewise sometimes be unreliable. As it is important to use for the comparisons only such body-parts as are well delimited, we will confine our comparisons to legs, wings and tergites.

We are thus going to compare the relative frequency of legs, wings and tergites which are of mixed male and female nature. In the tables

TABLE 1. *Number of selected body-parts of mixed male-female nature from 140 gynandromorphs of the present study (new series) and from 213 gynandromorphs from* PATTERSON *and* STONE *(1938).*

	1st legs	2nd legs	3rd legs	total legs	tergites	wings
PATTERSON and STONE						
Number of body-parts..	10	1	0	11	14	27
Percent of total number of body-parts	2,35	0,23	0	0,86	0,66	6,34
Own material						
Number of body-parts..	21	6	2	29	57	10
Percent of total number of body-parts	7,50	2,14	0,71	3,45	4,07	3,57

of PATTERSON and STONE (1938) such parts are denoted by ±, and a direct comparison is thus possible. The comparison may now be made in two different ways. In Table 1 we have listed the total number of each of these *body-parts* and expressed the figures as percentages of all corresponding parts within the described specimens. For instance, in our material of 140 specimens there were found 29 legs of mixed male and female nature. Thus, as 140 animals have 840 legs, 3,45 percent

TABLE 2. *Number of animals among 140 gynandromorphs from the present study (new series) and among 213 gynandromorphs from* PATTERSON *and* STONE *(1938) which showed mixed male-female nature on selected body-parts.*

	legs	tergites	legs + tergites	wings	legs + wings
PATTERSON and STONE					
Number of animals	11	10	19	25	34
Percent of total number of gynandromorphs observed	5,16	4,69	8,92	11,74	15,96
Own material					
Number of animals	22	31	44	10	29
Percent of total number of gynandromorphs observed	15,71	22,14	31,43	7,14	20,71

of the legs were of mixed nature. Some specimens had more than one part of mixed kind, and in Table 2 is listed the total number of *animals* with mixed parts and these are expressed as percentages of the total number of animals described. The 29 mixed legs in our 140 specimens (Table 1) were, for instance, found on 22 animals (Table 2), and the 29 legs and 57 tergites (Table 1) were found on 44 animals (Table 2).

Whichever way the comparisons are made they show that we found more bisexual legs and tergites but fewer bisexual wings than PATTERSON and STONE. In the case of wing comparisons the difference is not absolutely statistically significant, P being of the order of magnitude 0,10 and 0,14 in Tables 1 and 2 respectively. We think it, however, not at all improbable that some wings of mixed nature have escaped our attention, so that our figures for that body-part may be too low. With regard to legs and tergites the differences are strongly significant. Now, one can, of course, never be quite certain that a minimal area of one sex surrounded by the opposite sex will not be overlooked. Hence, the relative number of mixed wings from PATTERSON and STONE and the relative numbers of mixed legs and tergites from our material are

only lower limits of the true relative numbers of these body-parts of mixed nature.

We have made the above comparisons in order to show that cases with mixed body-parts are relatively abundant. But this is not the whole story. PATTERSON and STONE emphasize that in several of their cases an area of female tissue was located within male tissue, and that

TABLE 3. *List of some of the most striking cases of gynandromorphs with very small female areas surrounded by male area. All cases are from our new series in which the gynandromorphs occurred after crossing irradiated wild males to y w sn females. Female areas are thus always wild type.*

Record No.	Description of the mixed areas in question
A 63	Right side of thorax including legs, *y sn* except one wild type hair on femur of 1st leg
B 69	Right tergites *y sn* except an area on 2nd tergite including 4 hairs which were wild type
B 85	4th and 5th right tergites *y sn* except a small area on the caudal side of the 4th tergite, which included 2 hairs of wild type
B 93	Coxa and femur of 1st left leg *y sn* except one wild type hair on each of these joints
C 12	Right side of thorax, including legs *y sn* except a few wild type hairs on femur of the 1st leg
C 65	All tergites *y sn* except a small area on 5th left tergite including 2 wild type hairs
D 58	1st left leg *y sn* (with sex comb) except 2 wild type hairs on femur

this can neither be explained by late elimination nor by somatic non-disjunction. Somatic segregation or crossing over (STERN, 1936) can evidently not be responsible for such cases. In our material we have several cases in which a very small female area was isolated within a male area (and also the converse, though not so often). Seven of the most striking cases are listed in Table 3. To these may be added at least six specimens with a white eye in which was located a red spot containing only a few facets. These were, however, usually situated close to the margin of the eye and thus it cannot be ascertained whether these female areas are wholly situated *within* male areas. Several other but less drastic cases could be added.

Before trying to make any conclusions from these findings a few

words should be said about the sex combs. These combs, which are situated at the distal end of the first tarsal joint of the males' forelegs, are regarded as unfailing secondary male characters. To distinguish with certainty between the colour of the combs on yellow and wild males seems to be practically impossible, since the combs are always dark even on yellow males and since the »gray» colour of wild flies is in fact yellowish. No difference in sex comb structure can be observed

TABLE 4. *Gynandromorphs with sex combs surrounded by, or situated in the vicinity of, female areas.*

Record No.	Description of cases
A 29	The whole of thorax wild type but a small though distinct sex comb on right foreleg
C 13	Right sternopleurals, mesosternal, legs and wing wild type but a small though distinct sex comb on right foreleg
A 84	A few hairs on each of coxa, femur and tibia of the left foreleg *y sn*, the rest of this leg wild type but with sex comb
B 53	Coxa and trochanter on both forelegs *y sn*, femur partly *y sn* and partly wild type. The rest of the legs wild type but with sex combs on both forelegs
B 75	Left legs wild type with the *possible* exception of a few *y sn* hairs on the 1st tarsal joint of the left foreleg. A small sex comb on this leg
B 91	Left legs wild type except a few *sn* hairs on femur of foreleg (this gynandromorph occurring after elimination of a broken *X* chromosome was not *y* on male parts but *w sn*). Other parts of left foreleg, including tarsus, were as far as could be seen wild type. The leg had a small but distinct sex comb
B 93	Left thorax wild type, except parts of foreleg (see Table 3). This leg had a small but distinct sex comb

between singed and wild males. We refrain, therefore, from trying to state the nature of the sex comb itself in our cases of mixed first legs. But we have a few specimens in which either sex combs have been found within an otherwise female surrounding or in the vicinity of female tissue (Table 4) and also specimens which lack sex combs in spite of vicinity to male tissue. We must thus conclude that either the underlying and therefore not observable tissue is female when the sex comb is lacking and male when it exists or that the sex of the neighbouring tissue may exert some influence (hormonal?) on the development of the sex comb. Because of the fact that the sex combs we found

on wild legs are smaller than normal we think that the latter conclusion is more probable.

Returning now to the cases of small female areas within male surroundings (Table 3), the possibility of influence from underlying tissue or from tissue lying somewhat more apart cannot wholly be denied. We find, however, such an explanation rather improbable. If, for instance, the single wild type hair on the first right leg of specimen A 63 (Table 3) were due to influences from female parts, these influences must have been exerted from parts situated relatively far away, as the whole right side of the thorax, apart from the hair in question, was *y sn*. If an underlying female tissue were responsible, it must have been of very small dimensions, as otherwise more of the observable tissue, by the same kind of influence, ought to have been of female nature. This specimen should thus, if the nature of the underlying tissue were responsible, still be a specimen with a very small female part situated as an enclave within male tissue, and this enclave would consist not only of observable but also of non-observable underlying tissue. We think therefore that the most probable conclusion to be drawn from the relatively frequent occurrence of patches of female tissue surrounded by male tissue in gynandromorphs is that a study of the distribution of male and female parts and of their proportions is not relevant to the question concerning the cleavage at which the elimination occurs.

From studies of the early cleavages and the later embryonic development in *Drosophila* (HUETTNER, 1923, 1924; CHEN, 1929; PARKS, 1936) the following two facts are known. (1) All early nuclear divisions (apart from those in the germ line) are synchronous up to blastoderm formation; (2) the development is indeterminate so that chance movements of the early nuclei determine from which cells the descendants shall form any specified part of the body surface. When elimination occurs at the first cleavage, exactly half of the nuclei will be male and the other half female as long as the divisions are synchronous. After a certain stage this synchronism comes to an end, which, thanks to the indeterminate development, makes it possible that sometimes the female area is larger than the male area and sometimes the converse is true. On the average, however, the gynandromorphs should be expected to be $^1/_2$ male and $^1/_2$ female if the elimination always occurs at the first cleavage division, and if no other factor tends to disturb the synchronism. But this synchronism, it must be remembered, has been found in studies of the normal development. SONNENBLICK (1940), who studied the cytology of eggs developing after X-ray irradiation, found a number

of more or less severe disturbances. It seems probable that the elimination process, of whatever nature it might be, usually retards the formation of the descendants of the eliminated ancestral nucleus in relation to those of the uneliminated ancestral nucleus. This would then account for the fact that not only after irradiation, but also in spontaneous gynandromorphs, the female area is usually greater than the male area (PATTERSON and STONE, 1938). The chance movements of the early nuclei makes it further natural that in the majority of cases the descendants from each of the two nuclei of the 2-nuclei stage keep more or less together, thus forming in the imago two distinct areas, one female and one male. In a minority of cases, however, these two areas will be mixed and in individual cases some few female cells will happen to lie intercalated within a male area.

2. THE ELIMINATION PROCESS.

In the earlier studies it was believed that the *X* chromosomes could be eliminated simply through lagging on the spindle (MORGAN and BRIDGES, 1919). That sometimes this may really be the cause of spontaneous gynandromorphs cannot be excluded. If suitable marker genes are used specimens will be found now and then among gynandromorphs produced by X-ray irradiation in which only part of the *X* chromosome is eliminated. PATTERSON and STONE (1938) concluded that the *X* chromosomes of the irradiated spermatozoon in such cases must have been in a two-strand stage and that only one of the chromatids was broken. But whether or not the gametic chromosomes are in a two-strand stage at the moment of irradiation, this does not give any clue to the *causes* of elimination. According to MULLER (1940), mosaicism produced by irradiation of the male is due to breakage of an unsplit spermatozoon chromosome, and that after splitting one of the chromatids fails to reunite. If PATTERSON and STONE's assumption is correct (and if no other complementary assumption is made), the male parts of a gynandromorph should always be of one and the same genotype. They also stress that in their material, so far as could be determined, two separate areas of male tissue always are of the same genotype. Among our specimens, however, we found two cases with different genotypes in different male parts. These cases were as follows.

Specimen III 250. — From a cross between *y w f Bx* females and irradiated wild males (old series). The whole head and the right side of thorax and abdomen were wild type and female. On the left side the thorax was forked but not yellow (as mentioned in the introduction,

our descriptions were not so thorough and detailed in the old series as in the new one, and the genotype of the legs of the left side is uncertain; there was, however, a sex comb), left wing probably forked and much Beadex (*Bx* is dominant but with incomplete penetrance) but not yellow. Left side of abdomen was yellow and forked and the genitals mostly male. There were thus two male areas of different genotypes, *viz.* yellow forked and forked.

Specimen C 19. — From a cross between *y w sn* females and irradiated wild males (new series). The whole of the right side of the animal was wild type and female. The left eye was white as was the anterior ocellus. The two posterior ocelli were red. On the left side of the head the following bristles and hairs were singed but not yellow: vibrissa, carina, some hairs ventrally to the carina and the two verticals. The left palpus was likewise singed but not yellow. The rest of the left part of the head was wild type and female. On the left side of the thorax the following bristles were yellow and singed: upper humeral, supra-alars, dorsocentrals and the 3rd leg. The rest of the left side of thorax was singed but not yellow and the foreleg had a sex comb. The left side of the abdomen (tergites) was singed but not yellow and male coloured. Genitalia mixed. This specimen, thus, showed on its male parts the two genotypes yellow singed and singed.

Supposing the theory of the spermatozoon chromosomes being in a two-strand stage to be correct, the additional assumption of double elimination, one from the X-ray chromatid break and one spontaneous at a later cleavage division, could explain our two cases. The probability of observing two cases of double elimination in our material seems, however, to be small. If the number m denotes a common relative frequency with which eliminations are observed, the expected proportion of animals in which more than one elimination has been observed will be $1—e^{-m}—me^{-m}$, which, for small values of m, is equal to $\frac{m^2}{2}$. It is to be noted that m does not denote a true frequency but the frequency which can be observed with the observational capacity of the observers. The frequency of observed gynandromorphs was about 2 per 10^4 in sub-series III of the old series and about 6 per 10^4 in the sub-series C of the new series. (As pointed out in our previous paper, many gynandromorphs with only small male patches certainly escaped attention in the old series, and changes in the observational capacities of our co-workers during the course of the experiments was the reason why no statistical analysis was made of dependence of frequency of

gynandromorphs on dosage.) In the old series the frequency of observed spontaneous gynandromorphs was about 0,6 per 10^4; in the new series no experiments were made giving a corresponding frequency. In order not to choose m too small we may suppose it to be 7×10^{-4}, which certainly is grossly exaggerated. Our total material of females and gynandromorphs in the old and the new series is somewhat short of 1,400,000. We should thus expect to find $\frac{49}{2} \times 10^{-8} \times 1{,}4 \times 10^6$ animals with double elimination, *i. e.* 0,35 such animals. χ^2 in this case is 8, corresponding to a P between 0,01 and 0,001. Although at such small expectations the χ^2 test very much exaggerates the discrepancy between observation and expectation, we think that the high value of m which we have chosen makes the conclusion valid that the process which in the two specimens leads to the formation of the male area of the one genotype is not independent of the process leading to the male area of the other genotype. Or, in other words, if one of the eliminations is due to the irradiation, then it seems to be very probable that the other elimination also is due to the same cause. If this conclusion is accepted, our two specimens form a strong argument against the validity of PATTERSON's hypothesis.

Thus, following MULLER (1940) and PONTECORVO (1942), the production of X-ray induced gynandromorphs seems best to be explained as follows. By the irradiation an unsplit spermatozoon X chromosome is broken. The broken ends remain in this condition until chromosome splitting. After splitting one of the chromatids restitutes, whereas the cut-off piece of the other chromatid is lost (or translocated). The fertilized egg nucleus thus contains one untreated maternal chromosome and one treated paternal chromosome in which one chromatid is undeleted and one deleted (Fig. 1). At the 2-nuclei stage one of the nuclei then contains 2 normal X's the descendants of which form the female part of the gynandromorph. When the deleted chromosome of the other nucleus of the 2-nuclei stage splits, MCCLINTOCK's (1938) principle of fusion-bridge-breakage starts with the probable ultimate result of elimination. The first bridge to form, thus, does so during the second cleavage division. In the resulting 4-nuclei stage there may be either two nuclei with complete elimination, or one nucleus with complete elimination and one with a broken X chromosome, or two nuclei with broken chromosomes. In the 8-nuclei stage there will be one to four nuclei with complete elimination, and so forth. It can thus not be predicted when the actual complete elimination occurs in the different cell

lineages. But it may be assumed that the closer to the centromere the original break was situated the smaller is the number of cleavages before complete elimination (McCLINTOCK, 1938). It is perhaps conceivable that a broken *X* chromosome persists in a few cell lineages until the imago is completed. These cells should be hyperploid male if the remaining part of the chromosome is small. If, as according to SUTTON (1941), broken chromosomes in *Drosophila* may sometimes heal (as in the case of maize; McCLINTOCK, 1941), the possibility of such male cells

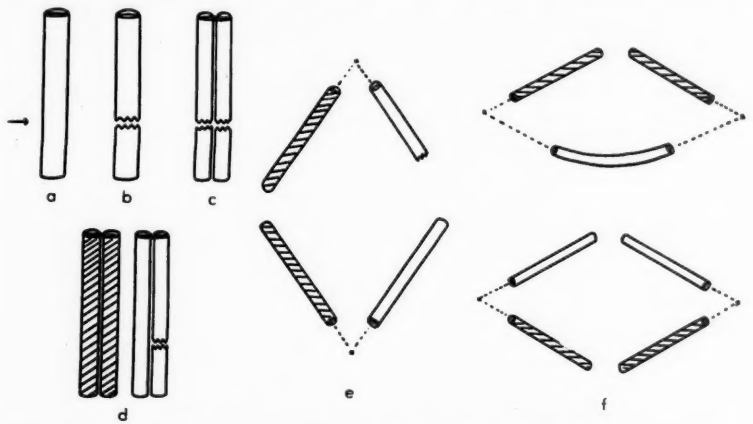

Fig. 1. Paternal *X* chromosome (unstriped) containing normal genes; maternal *X* chromosome (striped) containing recessive genes. — *a*, hit; *b*, break; *c*, split; *d*, fertilized egg nucleus with one chromatid restituted and one unrestituted; *e*, anaphase of 1st cleavage division; *f*, anaphase of 2nd cleavage division. From one of the nuclei (upper row) the fusion-bridge-breakage cycle starts, leading ultimately to elimination and formation of male cells. Female cells are formed from the other of the nuclei.

with broken non-eliminated chromosomes occurring in the imago is increased. It should here be remembered that the break-fusion-bridge process often leads to dominant lethality (PONTECORVO, 1942) and that this possibly may be due to the occurrence of persisting bridges uniting the two daughter nuclei to one nucleus and thus producing polyploid nuclei (DEMEREC and FANO, 1944). In the development of the actually observed gynandromorphic specimens, however, dominant and fully lethal chromosomal aberrations could of course not have been formed.

In the case of gynandromorphs containing in their male parts broken *X* chromosomes (*i. e.* not broken as residues of a breakage-fusion-bridge cycle but broken from the very start) it is not necessary

to suppose that the breakage-fusion-bridge mechanism ever comes into play. For instance, when, as in our new series, a few gynandromorphs are found which in their male parts are *sn* (or *w sn* if the eyes are male) but not *y*, these may be explained to have occurred from a paternal *X* which was broken on either side of *sn*. If, after splitting, restitution occurs at both breaks in one of the chromatids whereas the middle piece of the other chromatid containing sn^+ is lost and the distal part containing y^+ or $y^+ w^+$ joins with the proximal part there will be no broken ends. Then, already at the 2-nuclei stage one of the nuclei has a paternal *X* lacking the gene sn^+ but containing y^+. This nucleus will thus be the

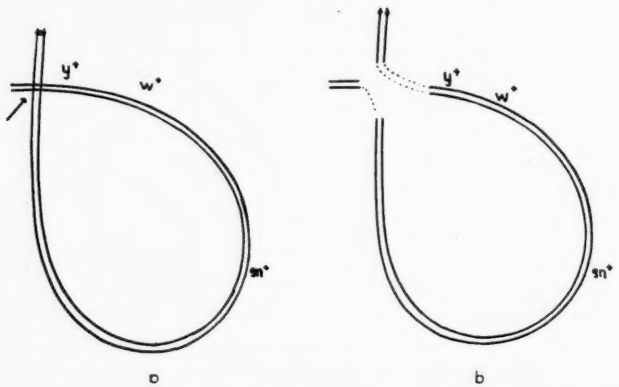

Fig. 2. — *a*, split chromosome forming a loop and hit at the point of intersection; *b*, restitutions (indicated by dots) forming one complete chromosome and one chromosome lacking the distal part and having the genes $y^+ w^+ sn^+$ in inverted order.

ancestor of the male parts. It would seem that the part of the irradiated *X* chromosome which is retained in the male parts must be rather small unless the unbalance of hyperploidy should make the animal inviable — or, indeed, produce a hypoploid female part instead of a hyperploid male part.

On the basis of this mode of production of gynandromorphs our two specimens III 250 and C 19 (p. 308) may be explained in different ways. C 19 may, for example, have been produced as follows (Fig. 2). The irradiated *X* chromosome was broken at two places, one distally to *y* and the other proximally to *sn* and probably close to the centromere. The piece between the breaks was inverted, for instance, by a loop in the irradiated chromosome. After having split one of the chromatids was wholly restituted (and it is, of course, irrelevant if this

was done in the inverted or in the original order). From this come the female parts. In the other chromatid the distal tip was lost whereas the part containing the normal alleles of *y w* and *sn* was attached in inverted order to the proximal part. By the breakage-fusion-bridge process this chromosome was eliminated from some of the male cells but in other such cells the now proximal piece containing y^+ was retained. Since a rather large part of the specimen was *sn* but not *y* it seems

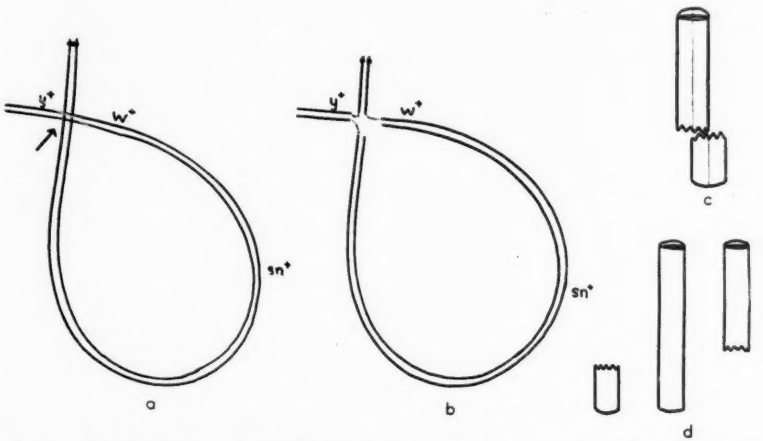

Fig. 3. — *a*, split chromosome forming a loop and hit at the point of intersection; *b*, restitutions (indicated by dots) forming one complete chromosome and one chromosome made up of the proximal and distal parts; *c*, the hypothetical irregular restitution in the short chromosome; *d*, results of split at next division.

probable that there was a healing of the y^+ tip (it should be remembered that the healings reported by SUTTON, 1940, occurred at the tip of the chromosome).

Another conceivable mechanism in action in the production of specimen C 19 — without either the assumption of double elimination or of healing — is the following: when the paternal chromosome was hit it lay in a loop, as shown in Fig. 3. After splitting, one of the chromatids restituted normally, giving rise to the paternal *X* chromosome of the female parts. If in the other chromatid the tip including the gene y^+ was attached to the proximal part but in a somewhat oblique or otherwise abnormal fashion the split and re-attachement could take place so as to produce one daughter chromosome with a broken end — giving rise to the *y sn* male area — and one short y^+

containing daughter chromosome without a broken end — giving rise to the *sn* (hyperploid) male area.

Some similar procedure may be thought to have occurred in the development of specimen III 250. But to state what actually happened in the production of these two specimens is impossible. It may perhaps be objected that neither of the two explanations which we have offered here is a more likely one than double elimination. To this we can only answer that the probability of double elimination may be approximately estimated and shown to be low, whereas we do not know the order of magnitude of the probability corresponding either to the first or to the second of our alternative interpretations of the procedure leading to the occurrence of the specimens III 250 and C 19.

III. PROGENY TESTS OF MUTANTS AND ABERRANTS.

As was indicated in the first part of the present studies (BONNIER and LÜNING, 1949), a number of mutants and other aberrants were progeny-tested by outcrossing to brothers. In the case of the old series the mothers (*i. e.*, the females with which the irradiated males were mated) were homozygous *w f* and the aberrant females were outcrossed to *w f* males, and in the case of the mothers being *y w f Bx* the females were outcrossed to *y w f Bx* males. Likewise, in the new series, in which all mothers were *y w sn*, the aberrant females were outcrossed to *y w sn* males. For reasons of different kinds the progeny was not actually counted in all cases, so that for a number of aberrant females it is only known what kind of types occurred among the progeny.

At the progeny-testing of the females it could, of course, not always be taken for granted that these females were virgin. However, as described in our outlines of the methods used in the old series, the irradiated males were mated to homozygous females for two to three days, after which the females were transferred to new bottles without males. The daughters of the irradiated males among which the aberrant females occurred could not therefore have copulated with their wild fathers. If they were not virgin, their non-virginity could thus only have been brought about by copulation with brothers. The bulk of these were of the type with which these females were to be mated (*w f* and *y w f Bx* respectively), and such non-virginity could thus not do any harm. Non-disjunctional wild males occurred among the brothers now and then, but as these must practically always have been due to primary non-disjunction they were sterile. Other types of males which also

occurred, such as *f* or *w f Bx*, which presumably also were *XO* males, must likewise have been sterile. In the sub-series C and D of the new series the irradiated males were left with the females until classification of the progeny began, and non-virginity of aberrant daughters could in these series be due to copulation with the wild fathers. In the sub-series A and B of the new series the irradiated males were not left with the females. Hence, in these two sub-series the same circumstances concerning non-virginity of the aberrants prevail as in the old series. In all series some of the wild-type sons of the irradiated males might have been due to secondary non-disjunction, and when such males copulate they may produce progeny. However, if the percentage of exceptions was not too low in these cases, non-disjunctional daughters (*i. e.*, *w f*, *y w f Bx*, *y w sn* respectively in the different series) should also occur among the progeny of the irradiated males. As this could also be due to non-virginity of the homozygous females with which the irradiated males were crossed, culture bottles in which such females were found were always discarded. Summing up we thus find that in the old series and in the sub-series A and B of the new series unexpected results in the progeny tests could hardly be due to non-virginity. This might, however, be the case in the sub-series C and D of the new series.

In mass experiments with *Drosophila* there is always a slight possibility of contamination in spite of the most careful scrutiny. It is thus not absolutely impossible that in very few cases a wrong female was placed in a culture bottle instead of, or together with, the female which was to be progeny-tested. Quite unexpected results might then occur.

Salivary gland preparations were made only very occasionally.

In the majority of cases the whole progeny of the mutants showed the mutation in question. In several cases the mutation was accompanied by lethality and/or crossing-over depression. These cases will not be dealt with here, only their numbers in the old series will be given. Instead, we are here going to enumerate cases of more interest in the present connection.

1. WHITE MUTANTS GIVING WILD TYPES OR NON-WHITE NOTCH IN THEIR OFFSPRING.

In the old series a total of 85 white mutants were progeny-tested (in Table 2 of our previous paper this figure is given as 83, but in series I 12 and not 10 white females were tested). Of these 85 white mutations, 79 gave only white in their progeny (70 by actual counting,

9 by only stating the nature of the offspring). The remaining six gave, besides white, also wild type in the offspring; in the case of four of them the progeny was not actually counted (Table 5) whereas this was the case for the remaining two (Table 6 *a*, *b*). In the new series we have six white mutants which, when progeny-tested, gave wild types in the offspring (Table 6 *c*).

TABLE 5. *Number of progeny-tested white and forked females for which no actual counting of the progeny was made but the presence of wild type flies was noted.*

Parentage of females	Females mated to	white	forked
w f ♀ × irr. wild. ♂	*w f* ♂	3	3
y w f Bx ♀ × irr. wild. ♂	*y w f Bx* ♂	1	

When a white mutant occurring as in our experiments from irradiated males crossed to homozygous *w* females gives a number of + offspring on being mated to *w* males, this can theoretically be the result of two different causes (apart from non-virginity or contamination). The mutation may be a dominant. In such a case there would be expected 3 white : 1 wild if the dominant is autosomal or a smaller proportion of wild if the dominant is sex-linked. Or the mutant female may be a mosaic, either a gonadic mosaic (AUERBACH, 1947), her ovaries having one part mutated and one part non-mutated, or a gonosomic mosaic, her ovaries and her somatic tissue being of different genotypes. For the four cases enumerated in Table 5 we do not unfortunately

TABLE 6. *Progeny of white females and of a female with yellowish red eyes which yielded wild type in the progeny.*

(a) Progeny of a *w N* female with the parentage *w f* ♀ × irr. wild ♂ when outcrossed to *w f* males.

Record No.	females			males		
	N	*w N*	*w f*	+	*w*	*w f*
VII 103	2	2	3	1	3	5

(b) Progeny of a white female with the parentage *y w f Bx* ♀ × irr. wild ♂ when outcrossed to *y w f Bx* males. Progeny not classified with regard to *Bx*.

Record No.	females			males
	+	*y w f*	*w*	*y w f*
I 681	17	11	1 [1]	21

[1] This animal was probably not a daughter to I 681 but I 681 itself.

(c) Progenies of white females and of a female with yellowish red eyes (C 85) with the parentage *y w sn* ♀ × irr. wild ♂ when outcrossed to *y w sn* males.

Record No.		females						males						
		+	*y w sn*	*y w*	*s n*	*y*	*w*	+	*y w sn*	*y w*	*s n*	*y*	*w sn*	*w*
A 64	...	11	12	3	4		1 [1]	7	13		4			
A 97	...	7	11	2	3			8	4	1	1			
C 78	...	18					2	1	17	1			2	7
C 85	...	21	15	3	7	2		25	15		4	2		1
C 107	...	36	1				5	2	9	3				

[1] This animal was probably not a daughter to A 64 but A 64 itself.

know anything about the proportions between whites and wild types in the progeny. The only thing we can say is that dominant eye colours are infrequent, but this does not, of course, rule out the possibility that one or more of these four are dominants. The nature of VII 103 (Table 6 *a*), which was a white Notch, is difficult to determine because of the small number in the progeny, but the occurrence of Notch makes it certain that it is a mutation in the white region. The animal I 681 (Table 6 *b*) was most certainly a gonosomic mosaic. The single white female collected when counting her progeny was probably the female I 681 herself, and the progeny then shows that her ovaries were heterozygous for *y w f*. But the lack of wild males and of crossovers shows that also in the ovaries the irradiated *X* chromosome was changed in some way. It may be imagined that there was more than one break, one of which lay in the *w* region. After splitting, the chromatids re-attached in different ways, though in equal positions, the one giving a *w* mutation or white through position effect and showing in the eyes of the fly itself; the other giving a Notch lethal and a structural alteration preventing crossing over and showing its effect in the progeny. Notch daughters were outcrossed to *y w f Bx* sons and among their progeny they gave mottled females. Such mottleds were again crossed to their *y w f Bx* brothers, from which a stock of mottleds was raised. Salivary gland preparations were made but not analysed in detail; they showed, however, complicated structural changes.

Of the females in the new series which were white and had wild type in their progeny (Table 6 *c*), the offspring of C 78 is probably to be explained by non-virginity, if it is assumed that the two white females and the wild male are non-disjunctionals. A corresponding

situation could be supposed to explain to a certain extent the offspring of C 107. However, two of her white and two of her wild daughters were again outcrossed to *y w sn* males from stock and salivary gland observations were made on the resulting larvae. It was then found that when the mother of the larvae was white there was a translocation between *X* and 2L with the *X* break in 3C, *i. e.* in the *w* region. When the mother of the larvae was wild type the *X*'s were quite normal (in both cases, however, it was found that part of 2R was unpaired and contained an inversion, but this had evidently nothing to do with the white mutation). This makes it probable that C 107 was a gonadic mosaic, the larger part of the ovaries containing a cytologically normal paternal *X*, and a smaller part the paternal *X*, 3 translocation. The cytologically normal paternal *X* chromosome must be supposed to act, in hemizygous condition, as a semilethal. (Compare the hypothesis of LEA and CATCHESIDE, 1945, that recessive lethals induced by irradiation but without observable chromosomal alterations are due to restitutional breaks.) The paternal *X*, 3 translocation also acts as a lethal in hemizygous condition. Together with another *w*-carrying *X* chromosome it gives the white phenotype. The female C 107 must be supposed to carry, in her eyes, this *X*, 3 translocation. But the high numbers of wild and the very low numbers of *y w sn* among her female progeny are not explained by these assumptions. We must suppose that C 107 was first fertilized by a wild male, and thereafter either fertilized by a *y w sn* male or not at all. In the latter case the single *y w sn* female was due to non-disjunction.

The female C 85 (Table 6 *c*) had yellowish red eyes, but this colour did not return in the progeny. She must be a gonosomic mosaic. Her progeny does not fit very well with the supposition that her ovaries were normally heterozygous *y w sn*/+. But in the absence of salivary analysis it is impossible to say whether she also was a gonadic mosaic. The two females A 64 and A 97 (Table 6 *c*) seem to have been normally heterozygous *y w sn*/+ in their ovaries, and they were thus gonosomic mosaics.

2. FORKED MUTANTS GIVING WILD TYPES IN THEIR OFFSPRING.

In the old series a total of 81 forked mutants were progeny-tested. Of these, 78 gave only forked in the progeny (72 by actual counting, 6 by only inspecting the progeny). 3 mutants gave wild type among the progeny (Table 5), all from inspection only. None of these cases are thus possible to analyse further.

3. THE PROGENY OF FRACTIONALS.

The word »fractional» of a mutant is here used in its customary meaning. A fractional white *female* is thus a female, *i. e.* an animal with female chromosomal equipment in all her cells, with eyes which are only partly white (including one white eye and one eye of other colour). Likewise, a fractional forked *female* is a female which is only

TABLE 7. *Progeny-tested fractional white females for which no actual counting of the progeny was made but the presence of wild type flies was noted in the progeny.*

Record No.	Parentage of females	Females mated to	Description of females
III 56	*w f* ♀ × irr. wild ♂	*w f* ♂	one eye white with small red sectors
V 23	»	»	left eye white with a small dorsal red spot
V 24	»	»	left eye white with a small caudal red spot
VII 377	»	»	left eye white
VII 422	»	»	half of one eye white
V 211	*y w f Bx* ♀ × irr. wild ♂	*y w f Bx* ♂	right eye white with caudal red sector

TABLE 8. *Progeny of fractional white females with the parentage w f ♀ × irr. wild ♂, when outcrossed to w f males.*

Record No.	Description of females	females		males	
		w	*w f*	*w*	*w f*
V 144	Both eyes white except for a few red facets in the left eye	22	22	22	21
VII 165	Left eye mostly white	46	53	15	43

partly forked. As pointed out in the first part of the present studies, a gynandromorph may be mistaken for a fractional. For instance when, in our old series, an only partly forked female with red eyes and normal, or seemingly normal, abdomen is classified as a fractional forked, this classification may in fact be erroneous if the animal is a gynandromorph. The same kind of error may also be made for fractional white, fractional yellow and fractional singed females, but certainly much less often. (It should in this connection be remembered that the forked allele which we used in our old series did not show well except on the bristles of head and thorax.)

TABLE 9. *Progeny of fractional white females with the parentage y w f Bx ♀ × irr. wild ♂ when outcrossed to y w f Bx males. Progeny not classified with regard to Bx.*

Record No.	Description of females	females								males						
		+	*y w f*	*y w*	*f*	*w*	*w f*	*y*	*y f*	+	*y w f*	*y w*	*f*	*w*	*w f*	*y f*
I 598	Left eye white with a red stripe. Left wing strongly *Bx* and perhaps smaller than normal	3	11	7	8	1			1	13	16	9	3	1		
I 610	Left eye white with a few red facets. Right eye white with red sectors. Wings strongly *Bx*	6	18	2	1	13				7	20	6	1	19		
I 629	Small white sector in left eye. Left wing *Bx*	8	2	7	3					6	9	3	4		1	1
III 236	Right eye white with two small red areas		6	4		8	4				11	5		2	5	
VII 428	One eye white with a small area of red facets		16	16		21	7				22	12		23	12	
X 2	Left eye with a stripe of white facets	19	7	10	6					12	10	4	3		1	
XI 32	Small white caudal area in right eye	12	10	9	5			1		10	7	5	8			

A. OFFSPRING OF FRACTIONAL WHITE FEMALES.

In Table 7 are listed all cases of F_1 females which in our old series were classified as fractional white females that gave wild types in their progeny when this fact was established by inspection alone. Tables 8, 9, 10 comprise all progeny-tested fractional white females with actual counting of the offspring.

TABLE 10. *Progeny of fractional white females with the parentage y w sn ♀ × irr. wild ♂ when outcrossed to y w sn males.*

Record No.	Description of females	females						males					
		+	*y w sn*	*y w*	*sn*	*w*	*w sn*	+	*y w sn*	*y w*	*w*	*w sn*	*y*
B 19	Left eye and left posterior ocellus white	17	24	4	4	23	6		27	7			
C 128	Left eye white with a few red facets. Left posterior ocellus white		27	5		28	6		22	7	2	3	
D 97	Dorsal portion of left eye, whole right eye and all ocelli white	29						4	8	4			1

As may be seen, the ovaries of the fractionals were wholly mutated (with or without lethality) in cases V 144 and VII 165 (Table 8), III 236 and VII 428 (Table 9), C 128 (Table 10). When yellow was used as a marker gene (Tables 9 and 10), and the ovaries were unmutated, only very few *y*, *y f*, *w*, *w f* could occur in the offspring. This was the case for I 598, I 629, X 2, XI 32 (Table 9) and D 97 (Table 10; this latter specimen was evidently not virgin). Except I 610 (Table 9) and B 19 (Table 10), the fractionals of Tables 8, 9, 10 were thus not gonadic mosaics but had either wholly mutated or wholly unmutated ovaries.

As for the females I 610 and B 19, they were at the same time somatic mosaics (fractionals) and gonadic mosaics. I 610 had presumably the larger part of her ovaries mutated and a smaller part unmutated. After white females that were descendants of I 610 of a later generation had been crossed to *y w f Bx* males it was found in the salivaries that region 1 of the *X* chromosome, perhaps excluding the very tip, was inserted between regions 7 and 8. Genetic tests showed the crossing-over percentage between *y* and *f* to be reduced to about 4,5 and this was the case for females heterozygous with respect to the

w-bearing mutated *X*'s as for those heterozygous with respect to the unmutated *X*, showing that both chromatids had undergone alterations.

B 19 (Table 10) was evidently also both a gonadic and somatic mosaic. That she was not the result of a dominant white mutation is shown by the lack of wild males in her progeny, which rules out the possibility of an autosomal dominant, and by the abundance of wild females among this progeny, which rules out the possibility of a sex-linked dominant mutation not allelic to white. Both of the paternal *X* chromosomes belonging to the different parts of her mosaic ovaries had a lethal action in hemizygous condition, one of them gave the wild, and the other the white phenotype. Her red right eye and her white left eye correspond to the two parts of the ovaries.

B. OFFSPRING OF FRACTIONAL FORKED FEMALES.

As pointed out above, females which were classified as fractional forked may in individual cases have been gynandromorphs and not fractionals. This might be the case with some of those listed in Table 11, where the offspring were not actually counted but it was only observed that wild types occurred among them. The same might also have been the case with regard to some of those enumerated in Tables 12 and 13. Compare, for instance, the note relating to III 322, Table 13. Another special case to be mentioned in this connection is VII 263 (Table 12). Among the progeny of irradiated wild males there rather frequently

TABLE 11. *Progeny-tested fractional forked females for which no actual counting of the progeny was made but the presence of wild type flies was noted in the progeny.*

Record No.	Parentage of females	Females mated to	Description of females
III 106	*w f* ♀ × irr. wild ♂	*w f* ♂	bristles on left side forked
V 59	»	»	bristles on left thorax forked
V 61	»	»	bristles on one side forked
V 71	»	»	bristles on right side forked
V 82	»	»	bristles on head and on left thorax forked
VII 143	»	»	a few bristles on thorax forked
VII 236 B	»	»	bristles on right thorax forked
VII 345	»	»	bristles on one side of thorax forked
VII 393	»	»	bristles on one side forked
III 273	*y w f Bx* ♀ × irr. wild ♂	*y w f Bx* ♂	bristles on right thorax forked
III 323	»	»	bristles on left side forked

occurred females with one or two of the thoracic bristles, usually scutellars, of the forked type. Several hundreds of these were outcrossed for progeny tests, but gave wild types among their offspring, and it was concluded that the bristle abnormalities were not due to a forked allele.

TABLE 12. *Progeny of fractional forked females with the parentage w f ♀ × irr. wild ♂ when outcrossed to w f males.*

Record No.	Description of females	females			males		
		f	*wf*	*w*	+	*f*	*wf*
VII 228	Bristles on left thorax except mesosternals forked	15	11	1	1	11	5
VII 263	One scutellar on right side forked		10			1	19

In one single case, VII 263, the whole progeny were forked (see Table 12), but, as of the 30 offspring, 29 were white forked and only 1 forked, contamination cannot be excluded. Except for this specimen, all females included here as fractional forked showed a somewhat larger area with typical forked bristles. The selection of those specimens which were included in Tables 11, 12 and 13 could not therefore follow any precise principles. (It should be remembered that, just because of the difficulty of telling which animals really are fractional forked, these animals were not included in any of the statistical analyses of our foregoing paper.)

Within Tables 12 and 13 there are three specimens, *viz.* I 558, III 322 and XI 26, which had forked ovaries. The single white female and the single wild male in the progeny of VII 228 (Table 12) suggest that this specimen has mosaic ovaries, the larger part being mutated and the smaller part unmutated. The specimens I 574, I 685, K 24, X 12 and X 18 may all well be explained as having unmutated ovaries.

C. OFFSPRING OF A FRACTIONAL SINGED FEMALE.

In our new series one single fractional singed female (C 110, Table 14) was progeny-tested. The result shows her to have unmutated ovaries.

D. OFFSPRING OF FRACTIONAL YELLOW FEMALES.

Table 15 shows the offspring of three fractional yellow females from our new series. The ovaries of A 95 were mutated to yellow and to a lethal; these two genes may be identical, *viz.* a lethal *y*. Specimen

TABLE 13. *Progeny of fractional forked females with the parentage y w f Bx ♀ × irr. wild ♂ when outcrossed to y w f Bx males. Progeny not classified with regard to Bx.*

Record No.	Description of females	females							males						
		+	*y w f*	*y w*	*f*	*y*	*w f*	*y f*	+	*y w f*	*y w*	*f*	*w f*	*w*	*y f*
I 558	Right side of thorax and abdomen forked		11		26					10		11	2		
I 574	Head except left ventral hairs and right side of thorax forked	12	14	14	12	2			17	8	10	8			
I 685	Right side of head and right side of thorax except sternopleurals, mesosternals, legs and wings forked.....	3	3	4	5				10	6	6	7			
III 322[1]	Left posterior vertical, ventral hairs, right orbitals, humerals (both sides), left mesosternal, first leg (both sides) and tergites forked		24		28		1	1		9		12			
K 24	Right side of head and right thorax forked. Possibly some forked hairs on right side of abdomen	10	7	10	12		1		10	4	9	8			
X 12	Description lost, only record left: forked mosaic	9	7	3	6	1	1		9	7	3	6			
X 18	Head forked, right thorax except legs and wing forked.....	24	20	10	15				16	12	10	15		1	1
XI 26	Left posterior vertical forked. Left thorax except legs and wing forked. Right thorax except scutellars, sternopleurals, mesosternal, legs and wing forked		15		17			1		19		13			

[1] It was noted that a few of the bristles seemed to be somewhat more yellowish than normal, though this could not be determined with certainty.

TABLE 14. *Progeny of a fractional singed female with the parentage y w sn ♀ × irr. wild ♂ when outcrossed to y w sn males.*

Record No.	Description of female	females				males			
		+	*y w sn*	*y w*	*sn*	+	*y w sn*	*y w*	*sn*
C 110	Scutellars, posterior postalar and posterior dorsocentral on right side singed	26	22	7	3	28	18	4	4

TABLE 15. *Progeny of fractional yellow females of the parentage y w sn ♀ × irr. wild ♂ when outcrossed to y w sn males.*

Record No.	Description of females	females						males					
		+	*y w sn*	*y w*	*sn*	*y*	*w sn*	+	*y w sn*	*y w*	*sn*	*w sn*
A 95	Right side of thorax except sternopleurals and posterior scutellar yellow		9	3		7			4			
C 83	2nd, 3rd and 4th abdominal left segment yellow	26	17	1	7		1		15	2		
D 56	Right side of thorax except humerals, posterior dorsocentral and scutellars yellow. Right side of abdomen except part of 5th segment yellow	6	3	3		1		3	5	2	2	1

C 83 had her paternal *X* chromosome mutated to a lethal, possibly allelic to *y* but not giving yellow colour, or to some hemizygously lethal structural change. The ovaries of D 56, finally, seem to have been unmutated.

4. PROGENY TESTS OF GYNANDROMORPHS.

In the old series a number of gynandromorphs were progeny-tested together with their non-gynandromorphic sisters. Out of 149 specimens which were recorded as gynandromorphs by elimination of paternal *X* chromosome, 19 were progeny-tested (many more were outcrossed but were sterile). Because of the risk of losing outcrossed

specimens, we refrained, in the new series, where it was of special importance to get detailed descriptions of the gynandromorphs, from trying progeny tests. Of the 19 progeny-tested gynandromorphs, 12 with wild type in their progeny were tested without actual counting the offspring. It seems probable that their ovaries were unmutated. The same

TABLE 16. *Progeny tests of gynandromorphs with the parentage y w f Bx ♀ × irradiated wild ♂ when outcrossed to y w f Bx males. Classification is not made with regard to Bx.*

Record No.	Description of specimen	females						males				
		+	*y w f*	*y w*	*f*	*w f*	*y*	+	*y w f*	*y w*	*f*	*y*
I 550	Left 1st and 2nd tergites *y f*. Thorax *y f Bx*, sex combs on both forelegs..	9	19	19	13	1		23	12	12	20	
I 627	Left scutellars and dorsocentrals *y f*	4	8	5				6	3	3	4	
III 296	Right thorax except foreleg *y f*	6	5	14	15		1	9	5	12	6	
K 3	Left tergites *y f*	6	8	1	2			11	11	6	4	1

TABLE 17. *Progeny tests of gynandromorphs of the parentage w f ♀ × irr. wild ♂ when outcrossed to w f males. Classification is not made with regard to Bx.*

Record No.	Description of specimen	females			males		
		w	*w f*	*f*	*w*	*w f*	*f*
III 74	Head and thorax forked. Sex combs on both forelegs		all showing forked			all showing forked	
VII 88	White eyes. Left side of head and scutellars *f*	4	4		5	9	
VII 146	Right eye white and some bristles forked	31	23		18	14	

is the case with the four gynandromorphs, the offspring of which are given in Table 16. The remaining three cases are shown in Table 17. The progeny of III 74 were not actually counted but it was ascertained that all were forked. In the case of VII 88 and VII 146 it seems obvious that their ovaries were mutated white, in the case of VII 146 probably a lethal *w*.

The fact that as many as three of our progeny-tested gynandromorphs are also gene mutations seems to contradict the possibility that

this is an incident due to chance. It may therefore be taken to indicate that breakage and gene mutation are not only due to a common cause, ionization, but also that when a break restitutes, the result will, at least often, be a gene mutation. If this is true, it may be looked upon as an extension of the hypothesis by LEA and CATCHESIDE (1945) that lethals produced by irradiation in which no re-arrangement can be detected in the salivaries are the result of restitutional breaks. In this respect one of our (not progeny-tested) gynandromorphs from our new series, C 8,

TABLE 18. *Progeny of some mutants with unexpected progeny. All have occurred after crossing females to irr. wild males. Classification is not made with regard to Bx.*

Record No.	Mother of specimen	Specimen outcrossed to	Phenotype of specimen	females							males				
				w	*y w*	*y w f*	*y w sn*	*y*	*f*	*y f*	*y w*	*y w f*	*y w sn*	*f*	*wf*
III 235	*y w f Bx* ♀	*y w f Bx* ♂	white			47						54			
III 258	»	»	white			38						33			
III 259	»	»	forked			12			1			14		1	
III 266[1]	»	»	white			all						all			
III 270	»	»	white		6	6					6	5			
V 98	*w f* ♀	*w f* ♂	forked						3	31				2	19
V 166	*y w f Bx* ♀	*y w f Bx* ♂	white	1	11	4				1	2	7			
D 69	*y w sn* ♀	*y w sn* ♂	yellow		7		11	3			9		17		

[1] The progeny contained a total number of about 150.

may be mentioned. It occurred in a cross of a *y w sn* female with irradiated wild males. The right side of its thorax and abdomen were *y* and its right foreleg had a sex comb, the external genitalia were mostly male. The head was not *y* or *sn* but the eyes were of a bright vermilion-like colour. It thus seems to be possible that the break which led to gynandromorphism also led to gene mutation. Against these conclusions it may be said to be very improbable that among such a small number of progeny-tested gynandromorphs no fewer than three should have had their paternal *X* chromosome broken just in one of the regions of the two loci under study, *w* and *f*. If the specimen III 322 (Table 13) were in fact *y* as well as *f* and thus classified as a gynandro-

morph instead of as a fractional forked, there would have been found still one more gynandromorph with a mutated ovary.

5. PROGENIES OF DOMINANT SEMILETHALS.

In Table 18 are listed a number of cases in which the class corresponding to the mutant's own phenotype is exceedingly small or completely lacking in progeny. When the progeny gives exclusively the maternal characters as in the cases of III 235, III 258 and III 266, contamination may be suspected. When counting was begun of the progeny of III 258, this white female was, however, herself found in the culture bottle, but this does not, of course, quite eliminate the possibility of contamination. In the other cases in which not only the maternal phenotypes were found in the progeny, contamination is very improbable. The most plausible explanation seems to us to be that the irradiated chromosome had become dominantly semilethal.

IV. THE RELATION BETWEEN THE CAUSES LEADING TO THE PRODUCTION OF GYNANDROMORPHS, FRACTIONALS AND GONADIC MOSAICS.

In the foregoing pages we have studied a number of specimens with regard to their mosaic nature, and have distinguished between three kinds of mosaics, *viz.* gynandromorphs, fractionals and gonadic mosaics. If the interpretation is true which, by following MULLER (1940), we have made regarding their origin, it is seen that a common mechanism is at hand. By irradiation of males, *X* chromosome breaks are produced. When, after splitting, one of the chromatids of the broken *X* restitutes but the distal part of the other is lost (or translocated), a gynandromorph is produced, its male parts being formed of the cell progeny from the nucleus with the broken *X* (Fig. 1). Restitution is often followed by a mutation in the gene which was hit, or in neighbouring genes. When both chromatids restitute two different mutations may be the result (one or both may be below our observational capacity; *cf.* also PANSHIN, 1935 and AUERBACH, 1947), or one of the chromatid restitutions may be accompanied by a mutation, the other not. In these cases a fractional will be produced if, within the organ(s) in which the mutation(s) can be observed, the paternal *X* chromosome of part of the cells is inherited from one chromatid and that of another part from the other chromatid. If these organs should

happen to be the gonads they must, for analogous reasons, be fractionals, but their fractional nature can, of course, not be directly observed but may be proven by their progeny showing them to be gonadic mosaics.

From the studies of germ cell formation in *Drosophila melanogaster* by HUETTNER (1923) we know that the fertilized egg nucleus divides eight times, forming 256 nuclei which all seem to be undifferentiated. At this stage the nuclei begin to migrate: the majority to the periphery forming the blastoderm, a small number — 5 to 11 in HUETTNER's preparations — to the posterior polar plasm, there forming the primordia of the gonads. For reasons of pure chance the entire group of these primordial germ nuclei must, in the majority of cases, be the descendants of one of the two nuclei of the 2-nuclei stage. In a certain number of cases, however, they must be descendants of both of these nuclei. In the latter event the resulting animal will be a gonadic mosaic if a break and double restitution together with unequal mutation occurred in the ancestral spermatozoon chromosome. It is possible to make a rough estimate of how often this would happen. If we make the simplifying assumption that there are no essential movements of the nuclei before the migration in the 256-nuclei stage begins, then the direction of the common tangent of the two nuclei of the 2-nuclei stage will determine the possibility of producing gonadic mosaics. If the radius from the centre of the egg to one border of the polar plasm is drawn and likewise the radius to the other border, the tangent should lie within the angle of these two radii in order to make it possible for descendants of both of the two nuclei of the 2-nuclei stage to enter the posterior polar plasm. Now, from drawings of the developing egg (HUETTNER, 1923) it is seen that this angle is approximately $^1/_4$ of a right angle and consequently about $^1/_8$ of all fractionals should be gonadic mosaics.

Our material of fractionals and gonadic mosaics is too scanty to permit any inquiry into this problem, and our experiments were not planned for such a study. There is, however, also another way to attack this question. If the history of the production of mosaics, as pictured here, is true, it follows that the proportion among gynandromorphs which have both ovarian and testicular tissue must be the same as the proportion of fractionals which are gonadic mosaics. No thorough investigation of the nature of gynandromorph gonads in *Drosophila melanogaster* is known to us. DOBZHANSKY (1931) investigated 171 specimens of gynandromorphs produced by the claret gene in *Drosophila simulans* and found that 29, or 17 percent, had one ovary and one

testis. On the one hand, however, DOBZHANSKY made no histological examination but confined himself to a classification based on the outer anatomical structure of the gonads. It is hence not known whether oogonia could occur within a seemingly testicular gonad or spermatogonia within a seemingly ovarian gonad, and it is thus not known whether or not the above percentage of mixed gonads may in reality be larger. On the other hand, it is not at all certain that the situation in these *simulans* gynandromorphs due to a specific gene action can tell us anything about the situation in gynandromorphs produced by X-ray irradiation.

V. THE LINEAR RELATION BETWEEN X-RAY DOSE AND MUTATION FREQUENCY.

When discussing the mode by which X-ray induced gynandromorphs are produced we tried to show that, in spite of the female areas of these flies being on the average twice as large as the male areas, there is no need to suppose that the process leading to *X* chromosome elimination ever starts as late as or later than the second cleavage division. In other words, we do not find it necessary to postulate in this respect any kind of delayed action of the irradiation and, hence, from our points of view, the proportions between female and male areas do not form any obstacle to the assumption that the eliminations are *direct* consequences of the ionizing hits. In this way the processes of gynandromorph formation are brought into harmony with the pre-requisites of the target theory. However, the statistical analysis of the relation between dose and mutation frequency in the white and forked loci which we carried out in the first part of the present studies (BONNIER and LÜNING, 1949) gave a picture that was not exactly in conformity with these pre-requisites.

We found there that the frequency of mutations associated with the doses 727 *r*, 1454 *r*, 2181 *r* and 2908 *r* doubtless increased linearily with the dose, but when the linear regression lines were extrapolated to the dose 0 *r* (spontaneous mutations with which we included mutations following doses 8 *r* and 16 *r*) we arrived at expected frequencies which in some cases were statistically far beyond the observed frequencies. In the case of mutation from wild to white the expected spontaneous rate was 0,58 per 10^4 as against none observed in a total of 304,796. When mutations of wild to white and wild to fractional white were pooled, the corresponding figures were: expected 0,74 per 10^4 as against 2 ob-

served in the same total (0,07 per 10^4). These two differences between expectation and observation are highly significant. In the case of mutation from wild to forked the expected frequencies were again higher than the observed, being 0,48 per 10^4 as against 1 observed in the same total (0,03 per 10^4). Here the significance of the difference was uncertain, as different statistical methods gave different results. Estimation of the regression lines not only for the above four dosages but also for the mutation rate at dose $r = 0$ (8 r and 16 r incl.) disclosed a discrepancy between expectation and observation that was still very marked, but its statistical significance was not so pronounced as before,

TABLE 19. *χ^2 tests of linearity of dose-mutation rate relation from data of* SPENCER *and* STERN *(1948) and χ^2 tests of difference between observed spontaneous rate (0,0974 percent) and expected spontaneous rate according to the different linear regressions.*

Doses in roentgens included in the test	Linearity test P	Expected spontaneous rate according to regression (percent)	Test of discrepancy between observed and expected spontaneous rate P
25, 50, 150	0,70—0,50	0,1560	$<$ 0,001
25, 50, 150, 500	0,05—0,02	0,0966	0,95—0,90
25, 50, 150, 500, 1000	0,20—0,10	0,1107	0,30—0,20
25, 50, 150, 500, 1000, 2000 ...	0,05	0,0932	0,70
25, 50, 150, 500, 1000, 2000, 3000	0,10—0,05	0,0979	$>$ 0,99
25, 50, 150, 500, 1000, 2000, 3000, 4000	0,02—0,01	0,0774	0,05

P being of the order of magnitude 0,10—0,05 for mutations from wild to white (and 0,30—0,10 for mutations from wild to forked). As it seems quite legitimate to contrast the rates at different X-ray dosages on the one hand with the spontaneous rates (incl. 8 r and 16 r) on the other, we found it to be very difficult to escape from the conclusion that the linearity of the mutation frequency—dose relation no longer holds when rates for $r = 0$ (and $r = 8$, $r = 16$) are included in the comparisons.

In spite of the fact that so many investigations have been made concerning the relation between dose and mutation rate there are not many which are suitable for an adequate comparison in this respect. The recent investigation by SPENCER and STERN (1948) seems, however, to be suitable, as it was made on a sufficiently large scale and includes a number of low doses. They studied the rate of lethal mutations in

Drosophila melanogaster after X-raying wild males at the doses $r = 25$, 50, 150, 500, 1000, 2000, 3000, 4000, and, in addition to this, the spontaneous rate. From their analyses they concluded that the linearity of the relation between dose and rate also holds for these low doses. They emphasized in this respect especially the rates from the lowest up to $r = 1000$.

In order to compare we have made χ^2 tests of linearity from the data of SPENCER and STERN (1948), including first the rates at the doses 25, 50 and 150 r, and then successively adding the next higher dose until the whole range from 25 r to 4000 r is included. Parallelly with this we have for each of the regression lines so investigated made a χ^2 test of the discrepancy between observed spontaneous rate and rate of expected at $r = 0$ by extrapolating each of the regressions. The results of our calculations are put together in Table 19. From this analysis it emerges that the best linearity is shown when only the rates at 25, 50 and 150 r are included, but the corresponding regression line gives an expected spontaneous rate of 0,1560 percent, which is far beyond the observed percentage of 0,0974. The deviation is statistically highly significant, P being less than 0,001. A fairly good linearity is also found when the doses 25, 50, 150, 500 and 1000 r are included, and the corresponding regression line gives a good agreement between expected and observed spontaneous rate (note, however, that also in this case the expected rate is larger than the observed). When the doses from 25 r through 3000 r are included, the linearity lies on the verge of significant deviation, but corresponds to a close fit between expected and observed spontaneous rate. The linearity tests for all other combinations of doses give too large deviations to be considered here, and the corresponding fits between expected and observed spontaneous rates are therefore without interest. (It should be observed that the bad fit to linearity when all doses from 25 r to 4000 r are included does not depend on any »saturation» effect, as the observed rate at the highest dose lies above and not below the straight regression line.)

The result from this analysis of the data of SPENCER and STERN (1948) are rather erratic, but they indicate, at least to some extent, the same kind of discrepancy between expected and observed spontaneous rates as those we demonstrated in our previous paper. Though we do not pretend to have definitely proved that such a discrepancy really exists, it seems to us necessary to take its existence under consideration as a probability. We are, however, aware of the difficulty of interpreting it in biological terms. It is, on the other hand, easy to construct a

formula which quite formally fits to our findings. By using the following symbols: true spontaneous rate $= a$; spontaneous rate expected according to straight regression line $= b$; slope of regression line $= k$; constant to be used for fitting to the data $= c$; dosage in roentgens $= r$; mutation rate at dose $r = y$, then the formula

$$y = a + \frac{b - a}{1 + \frac{c}{r}} + kr$$

gives an appropriate function. For $r = 0$, y will be $= a$ and when r increases the function approaches asymptotically the straight line $b + kr$. The smaller c is, the faster y rises from the initial value a to the regression line. The third term is proportional to r, the second term is constant unless r is very small and it increases with increasing r quickly from 0 to the constant value b—a. The interpretation of the discrepancy between observed and expected spontaneous rate would thus follow from the interpretation of this term. Quite tentatively we may consider the following (rather diffuse) interpretation. In addition to the direct, one hit action of the ionizing X-rays, there is a secondary (chemical?) effect which with increasing r rapidly attains saturation or else a kind of equilibrium. To gain more knowledge about this question it would be necessary to make further large-scale studies on the mutation frequencies within the range from $r = 0$ to, say, $r = 100$. So far, we have made no attempts in this direction.

SUMMARY.

(1) The present paper deals with questions concerning the formation of mosaics of different kinds after X-ray irradiation of wild males of *Drosophila melanogaster*. The material consists partly of that presented in a previous paper (BONNIER and LÜNING, 1949) and partly of new material. A short discussion is also submitted of the findings in the previous paper concerning the relation between dose and mutation frequency.

(2) A study is made of 140 gynandromorphs occurring after crossing yellow white singed females to irradiated wild males, cases with small female (wild type) areas isolated within male areas having been especially investigated. It is concluded that the proportion between female and male areas is irrelevant for the study of when *X* chromosome elimination occurs. The reason for the female areas being on the

average twice as large as the male areas is thought not to be that a number of eliminations occur during later cleavages, but that the elimination process itself retards the development within the cell lineage from these eliminations and that, hence, the male parts will usually be smaller than the female parts.

(3) Cases of gynandromorphs with sex combs on wild-type forelegs are described and discussed.

(4) Following MULLER (1940), it is supposed that irradiation produces breaks in unsplit spermatozoon chromosomes, and that after splitting one of the chromatids restitutes, whereas the other is lost (or translocated). From the latter chromatid follows a breakage-bridge-fusion cycle, ultimately leading to elimination and thus to male tissue.

(5) Two gynandromorphs are described, each of which within its male area had two different genotypes. It is shown that the probability of double elimination is very small, and hence these cases are regarded as strong arguments against the theory that the chromosomes, when hit, are in the two-strand stage.

(6) Cases of gynandromorphs which from progeny tests are shown to carry mutant genes are taken as arguments for the assumption that breaks and restitutions are often accompanied by mutations. This is also borne out by progeny tests of full mutants and fractionals. It is supposed that when both chromatids restitute after breakage two different mutations may often be produced. A fractional results when different regions of the body carry the chromosome progenies of these two different chromatids. If the gonads include parts of both of these regions, a gonadic mosaic will result.

(7) The similar history of gynandromorphs and of fractionals indicates that there is the same proportion of gynandromorphs with mixed male-female gonads as there is of gonadic mosaics among fractionals.

(8) The findings of the first part of the present studies are discussed. The relation between dose and mutation rate was found to be non-linear when spontaneous mutations were included. As the probability that this was a mere coincidence is very small, the possible interpretation is made that X-ray irradiation has two effects: one direct ionizing effect which is linearily related to dose and one indirect (chemical?) effect which, however, with increasing dose rapidly attains

saturation, so that, unless the dose is very small, this effect only adds a constant amount to the mutation frequency.

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NAPHTHALENE ACETIC ACID IN THE ALLIUM TEST

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I. AIM OF THE STUDY. GENERAL PLANNING OF THE EXPERIMENTS.

IN 1939 one of us studied the effect on *Allium* root meristems of various synthetic phytohormones, among them naphthalene acetic acid. The main histological and cytological effects were briefly described. Special attention was directed to the occurrence of polyploid mitoses in the old cortical tissue. It was demonstrated that the polyploidy in this case was of the endomitotic type, and the inducing factor was thought to be the growth in cellular volume. It could not be decided at that time whether the phytohormone caused this chromosome doubling or only acted as a detector of a polyploidy already induced by the natural growth of the cortical cells. After that, spontaneous chromosome doublings of the same type were demonstrated in differentiated tissues of many plants, among them *Allium* (BERGER and WITKUS, 1946). It is quite evident that tissue differentiation often is normally connected with endomitotic processes. It seemed, however, worth while to take up the question once more and to make a careful re-examination of the cyto-histological effects of naphthalene acetic acid on *Allium* roots.

DUHAMET (1939 a, b, 1945) found a certain antagonism in the growth effects of colchicine and another phytohormone, *viz.* indoleacetic acid. The growth inhibition by colchicine was partially counteracted by certain concentrations of the growth substance. We decided to study this problem further. We wanted especially to investigate the interaction of phytohormone and colchicine in the c-tumour reaction and in the c-mitosis reaction. If certain concentrations of a phytohormone allow roots to grow in such concentrations of colchicine as normally check growth, we wanted to know whether this effect is due to an action exerted on the tension growth or on the mitotic growth. As is well known, the growth inhibition caused by colchicine is due to both these factors; the cells in the tension region cease to extend in length and grow equally in all directions, causing the so-called

c-tumours, and at the same time the mitoses of the meristem take the character of c-mitosis, *i. e.* each cell grows in size but does not divide. The situation is complicated by the fact that certain concentrations of phytohormones bring about morphologically similar swellings (c-tumours) as does colchicine.

After one *orientating experiment*, started on November 27th, 1948, the following concentration series of naphthalene acetic acid (hereafter abbreviated NA) was tested (*experiment* 2, started on December 9th, 1948):

Concentration No.	3	4	5	6	7	8
Mol/l	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
ppm	186,2	18,62	1,862	0,1862	0,0186	0,0019

Concentration No.	9	10	11	12
Mol/l	10^{-9}	10^{-10}	10^{-11}	10^{-12}
ppm	0,000·2	0,000·02	0,000·002	0,000·000·2

The experiment was arranged with one repetition. After 11 days' treatment ($^{20}/_{12}$) all bulbs were moved over to water, and the experiment was finished after a further 16 days ($^{5}/_{1}$ 1949).

Experiment 3 (started on December 23th, 1948) was intended to show the effect of one concentration of colchicine on the same series of concentrations of NA as in experiment 2. Two series of bulbs were set, one of which contained 300×10^{-6} mol colchicine. After 14 days' treatment ($^{6}/_{1}$ 49) all bulbs were transferred to water.

Experiment 4 (started on January 6th, 1949) was identical with the preceding experiment. In this second experiment, however, each series was repeated twice. Observations were made during 22 days.

Experiment 5 (started on January 20th, 1949) included a varying colchicine concentration within three constant concentrations of NA. 250, 200, 150, 100 and 50×10^{-6} mol/l of colchicine were dissolved in NA concentrations 5, 7, 11 and in pure water. The experiment was intended to elucidate the threshold region in colchicine of c-tumour and c-mitosis in the presence of NA in growth-inhibiting, growth-retarding and growth-promoting concentrations.

Experiment 6, finally, (started on February 17th, 1949) was intended to yield material for a study of the cellular and nuclear volume in c-tumours as compared with corresponding volumes in untreated roots. Five repetitions of one tumour-forming concentration of colchicine (200×10^{-6} mol/l), of NA (10^{-5} mol/l) and controls in water were

made. In order to facilitate the identification of comparable regions of the c-tumours and of normal growing roots all roots were graduated with Indian ink spots at the beginning of the experiment.

II. EFFECT OF NA ON THE ROOT GROWTH.

At the start of the experiments the end of the longest root of each bulb was marked by an ink spot on the tube. Since always rather uniform bulbs were chosen for these experiments there was no great difference between the length of the longest root and the average length of the roots. For practical reasons it is favourable to follow the development of the longest roots. All experiments containing complete series of NA concentrations were measured in this manner and the results were rather concordant, showing that our observations may be generalized.

Concentration 3 (*i. e.* 10^{-3} mol/l; hereinafter the molar NA concentrations will be designated only by the exponents) is strongly toxic. Long treatment kills the root tips. In no case was any growth noticed in 3. The roots treated with this concentration do no recover after transfer to water, but new roots grow out later. Nos. 4 and 5 inhibit longitudinal growth almost completely, but induce the very characteristic c-tumours (Fig. 1, *a*, *b*). No. 6 permits some longitudinal growth to occur, at the same time giving tumour growth. The c-tumours of this concentration are of a characteristic elongated shape (Fig. 1, *c*). Nos. 7 and 8 do not give tumours, but in them longitudinal growth is clearly inferior to that of the control (Fig. 1, *d*, *e*). In most experimental series they cause a distinct growth retardation (Table 1). Nos. 9 and 10 are concentrations between retardative and stimulative in effect. They give no persistent results, in some series a retardation is seen, in others some degree of stimulation. Nos. 11 and 12, on the other hand, are clearly stimulative of longitudinal root growth. In Table 1 all values from 11 and 12 are considerably higher than in the controls. Since no concentration lower than 12 was tested, the lower limit of the stimulation region was not attained in our experiments.

As an instance of the growth curves determined in our experiments Fig. 2 is submitted. It is based on daily measurements during 15 days of treatment in experiment 4. Concentrations 4—8 have a more or less retarding effect, 9—10 behave as the control, 11 and 12 yield a considerable stimulation.

The action on the growth of the leaves was only observed in pas-

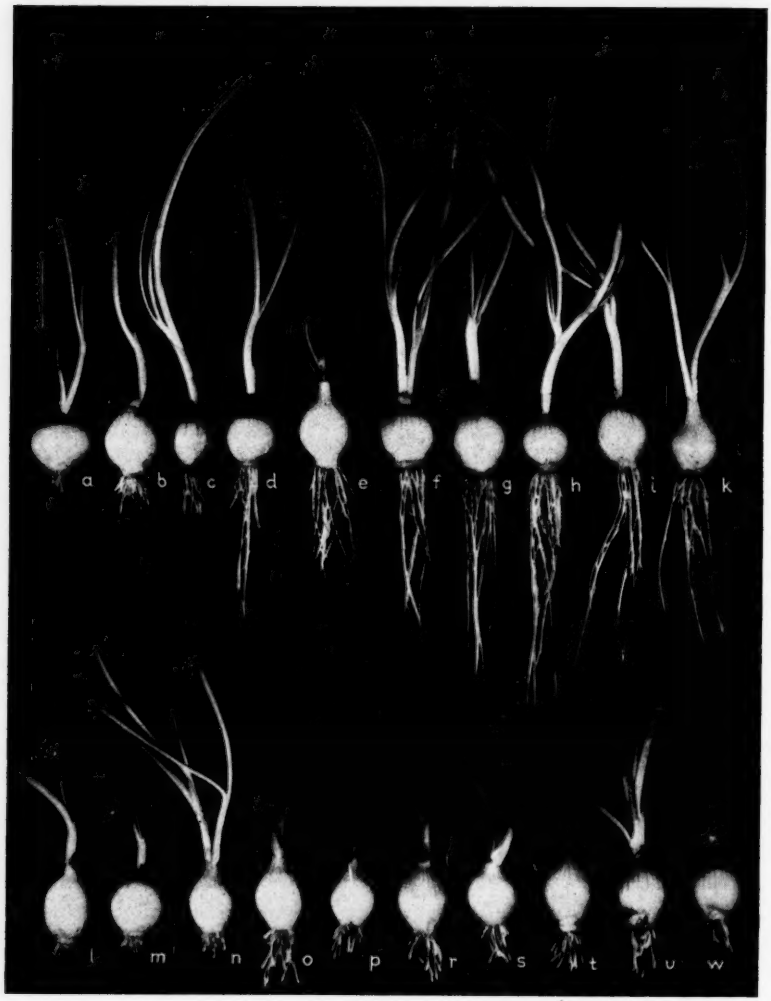

Fig. 1. The bulbs of experiment 4 after 13 days' treatment. *a—k*: NA 4—12 and control, *l—w*: the same NA concentrations + 300 × 10^{-6} mol/l colchicine. — Photo H. OLSSON, Svalöf.

sing. In experiment 4 some measurements were made. The growth in mm from the first measurement (day 3) to the last day of the experiment (day 22) was as follows (see page 342).

TABLE 1. *Root length (differences in mm controls—treated roots) in NA treatments in experiment 2—4.*

Concentration		3			4			5			6			7		
Experiment		2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
Day	1— 3	— 4	— 5	— 15	— 4	— 5	— 14	— 4	— 5	— 13	— 3	— 2	— 7	+ 4	— 4	— 2
	4— 6	— 32	— 38	— 46	— 31	— 37	— 45	— 29	— 38	— 43	— 18	— 28	— 30	— 3	— 23	— 20
	7— 9	— 51	— 70	— 65	— 51	— 69	— 64	— 49	— 70	— 62	— 25	— 51	— 42	— 5	— 38	— 25
	10—12	— 71	— 102	— 86	— 71	— 101	— 85	— 69	— 100	— 83	— 44	— 72	— 63	— 4	— 43	— 28
	13—15	—	— 117	— 98	—	— 116	— 97	—	— 112	— 95	—	— 82	— 74	—	— 42	— 26

Concentration		8			9			10			11			12		
Experiment		2	3	4	2	3	4	2[1]	3	4	2	3[1]	4	2	3	4
Day	1— 3	+ 3	— 1	+ 1	+ 3	— 1	+ 2	—	— 1	+ 7	+ 1	—	+ 4	+ 6	+ 2	+ 9
	4— 6	+ 2	— 5	— 14	+ 4	0	— 5	—	— 5	+ 11	+ 10	—	+ 4	+ 4	+ 5	+ 15
	7— 9	+ 4	— 24	— 20	+ 4	— 15	— 2	—	— 5	+ 9	+ 10	—	+ 19	— 2	0	+ 24
	10—12	+ 4	— 22	— 24	+ 5	— 16	— 1	—	0	+ 2	+ 20	—	+ 27	+ 12	+ 3	+ 29
	13—15	—	— 26	— 30	—	— 13	— 1	—	+ 3	+ 5	—	—	+ 28	—	+ 13	+ 31

[1] Died during the experiment.

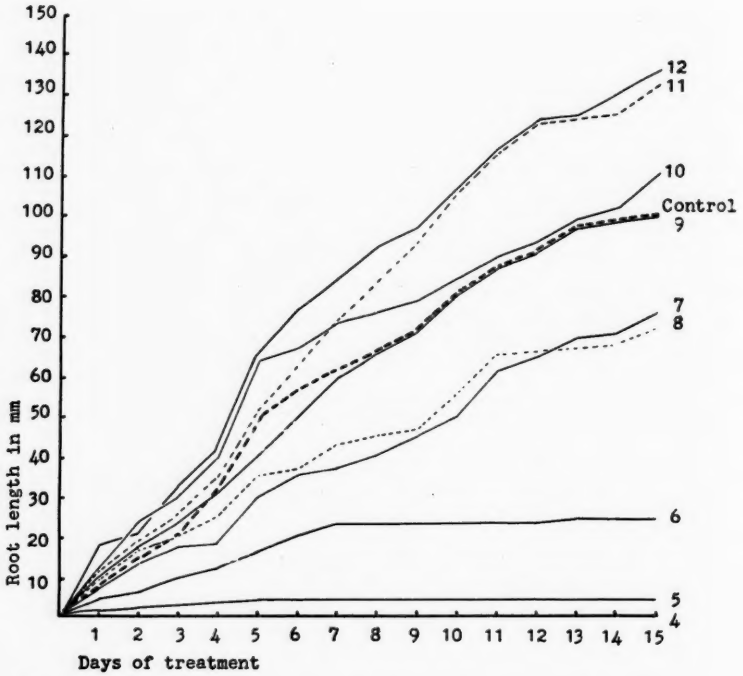

Fig. 2. Graph of the growth in length of roots in experiment 4, NA alone.

NA concentration number									
4	5	6	7	8	9	10	11	12	Control
264	272	345	330	320	290	269	318	255	310

The stimulative region seems to be situated at higher concentrations (6—8) than in the case of roots, a condition which is well-known from other works.

The effect of NA can accordingly be divided into the following zones:

3 strong toxic effect, growth inhibition

4, 5 growth inhibition } c-tumour formation

6, 7, 8 growth retardation (stimulation of leaf growth)

9
10
11
12 } growth stimulation

If the bulbs are transferred to water after some period of treatment, longitudinal growth gradually begins in the concentrations which have had a completely inhibiting effect (4 and 5). Those which have grown more slowly than the control under the treatment soon increase their growth rate and finally grow more rapidly than the control. This condition was observed in experiment 2, where the roots were studied during 16 days after the end of 11 days' treatment.

III. ROOT GROWTH IN PRESENCE OF COLCHICINE.

In experiments 3 and 4 the same NA series as in experiment 2 was tested together with a constant colchicine concentration. This was chosen at 300×10^{-6} mol/l for various reasons. It is well above the threshold value for c-tumour and c-mitosis ($\pm 100 \times 10^{-6}$ mol/l), and is still a concentration low enough to have little general toxicity in *Allium*.

Both experiments gave concordant results. As an instance, the bulbs of experiment 4 after 13 days' treatment are pictured in the lower row of Fig. 1, and the growth curves are given in Fig. 3. It is seen that in no case did the longitudinal growth exceed 9 mm during the 15 days of the experiment. As shown above, the controls of the same experiment grew 100 mm during the same time. It is clear that within an experiment in which the maximal growth is as small as 9 mm too great importance cannot be put on differences in growth between the various NA concentrations of that experiment. Some facts consistent of both experiments seem, however, to be characteristic. The control (colchicine + water) has an intermediate position. Those NA concentrations which without colchicine cause retardation, act similarly in the presence of colchicine, while stimulating concentrations also here bring about somewhat greater increase in length. On day 13, for instance, the situation in both experiments was the following:

Root length in relation to control		longer	equal	shorter
Experiment	3	8.11.12	10	3.4.5.6.7.9.
	4	7.8.9.10.11.12	6	3.4.5

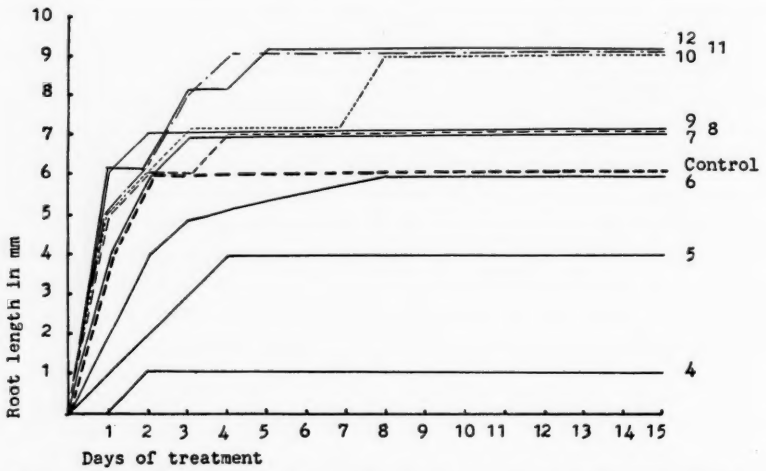

Fig. 3. Graph of the growth in length of roots in experiment 4, NA and colchicine.

All roots had c-tumours as expected. The tumours of 11 and 12 were clearly larger than those of 4 and 5 and of the control. This experiment thus confirms to a certain extent DUHAMET's result: The stimulating concentrations of NA counteract the growth-inhibiting effect of colchicine. The difference, however, is very slight, consisting only of a few mms. In all treatments where colchicine is present the longitudinal growth soon reaches its maximum, after which time no more growth occurs. The maximum is somewhat higher if a stimulating concentration of NA is present.

The growth of the leaves was followed in experiment 4. The increase in mm from day 3 to day 22 was as follows:

NA concentration number										
4	5	6	7	8	9	10	11	12	0	Water control
48	104	115	53	73	93	182	178	114	35	310

It seems evident that in this case NA counteracts the effect of colchicine, although conditions are more complex here than in the root tips. We have no measurements of leaves from the other experiments, but the results obviously followed the same direction.

The interaction between NA and colchicine is further studied in experiment 5, in which series of colchicine concentrations around the

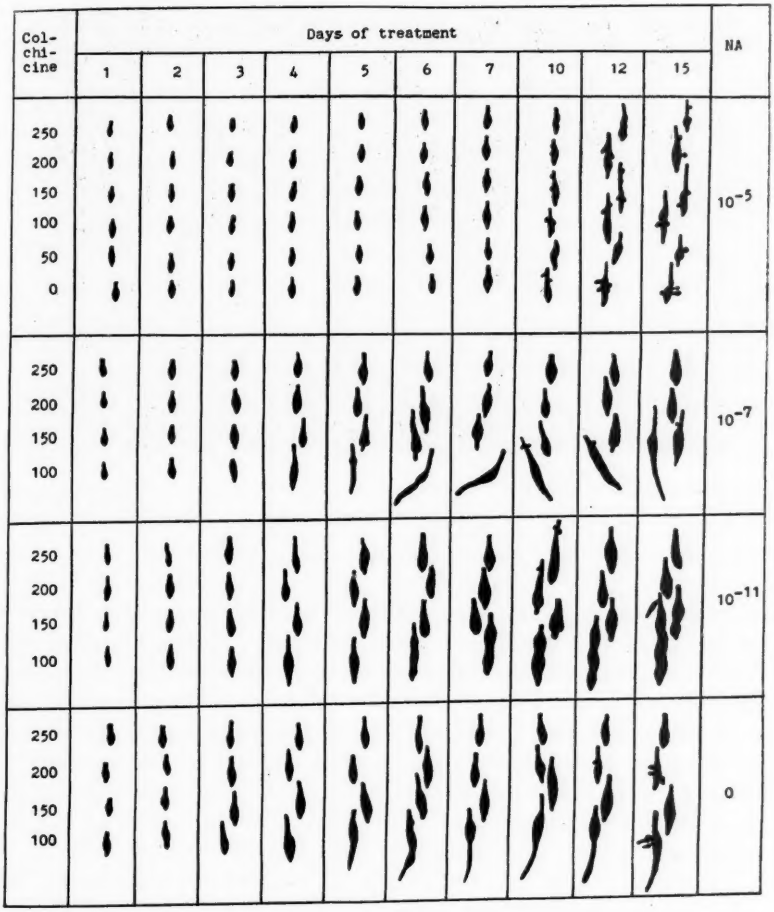

Fig. 4. Outline drawings of roots in experiment 5.

threshold value of c-tumours and c-mitosis are combined with NA of concentrations 5, 7, 11 and with water. The root length was observed during 15 days and the size and shape of the c-tumours were studied. Each day the outlines of one typical root of each concentration were drawn in scale. Fig. 4 is a survey of these drawings and Fig. 5 is a photograph of the four series on day 7.

Fig. 4 illustrates the development of the roots in this experiment

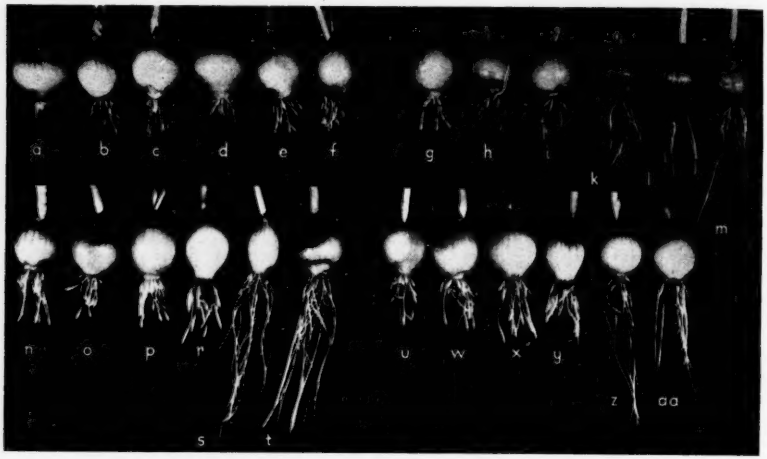

Fig. 5. The bulbs of experiment 5 after 7 days of treatment. *a—f*: NA 5, *g—m*: NA 7, *n—t*: NA 11, *u—aa*: water. In each of these four groups the colchicine concentrations are from left to right 250, 200, 150, 100, 50 and 0×10^{-6} mol/l. — Photo H. OLSSON, Svalöf.

better than words. All bulbs of the series with NA concentration 5 (the uppermost row of the picture) showed tumours. Earlier it was shown that NA of this concentration gave tumours. The addition of colchicine, thus, had no great effect. Possibly the higher concentrations effected some decrease in size of the tumours. A striking fact is the formation of lateral roots in this series. Starting on the 10th day it occurs even in such concentrations of colchicine as to all knowledge should be above the threshold value of the c-mitosis reaction. To this somewhat surprising fact we will return in the cytological treatment.

In the two other series of NA (Nos. 7, 11) as well as in water the weakest concentration of colchicine (50×10^{-6} mol/l) gives normal root growth without tumours. In the second lowest colchicine concentration (100×10^{-6} mol/l) there are tumours present which in all series have a tendency to growing out, the tumours being elongated. The three highest colchicine concentrations all give rather well-defined tumours. Since the concentrations of NA are only few although representative of the various action-zones of the substance, no definite conclusions can be drawn concerning the interaction of NA and colchicine in the c-tumour reaction. It can only be stated that our experiments do not indicate any additive or subtractive effect of the two substances on each

other. The threshold value is between 50 and 100×10^{-6} mol/l colchicine and between 10^{-7} and 10^{-5} mol/l NA. If there had been an addition of effect it would have been expected that 10^{-7} mol/l NA would have given tumours also in the colchicine concentration 50, and, on the other hand, if there had been any counteraction between the two substances, the colchicine threshold should have been heightened in the presence of NA. It may be mentioned here that conditions are similar in respect of the c-mitosis reaction. This situation may indicate that the c-tumours of NA and of colchicine are induced by different mechanisms.

BAUCH (1946) arrived at this conclusion in his experiments on the antagonistic effect of sulfone amide on the c-tumour reaction. Sulfone amide increased the threshold of c-tumours induced by colchicine, but did not influence those of phytohormones. The author concludes: »Wuchsstoffkeulen und Colchicinkeulen müssen also auf verschiedenen Stoffwechselvorgängen beruhen» (*l. c.*, p. 26).

As seen in Fig. 4 and also to some extent in Fig. 5, there is, however, a very striking difference in the size of the c-tumours of the different series of NA. The tumours are smallest in NA 5, somewhat larger in 7, but strikingly largest in 11. This was already noticed in experiments 3 and 4 but is very evident in the present experiment. While the differences in volume of the tumours were pronounced the differences in length were less clear. On day 15 the following growth in length from the beginning of the experiment was recorded:

Colchicine		250	200	150	100	50	0
	5	4	6	7	9	7	8
NA	7	11	10	10	28	83	101
	11	15	11	15	29	90	116
Water		10	14	15	28	89	110

On the 17th day the roots were cut off at the upper limit of the tumours and at corresponding points of the normal roots. Fresh-matter weight and dry-matter weight were determined. The latter values are given in Table 2. In spite of the considerable experimental errors the values clearly show an increase in dry-matter production of NA 11, while 7 and 5 give a decrease. Increased colchicine concentration consistently effects a decrease in dry-matter production. It is, thus, evident that even the low colchicine concentrations of this experiment exercise a distinct toxic effect.

It is of some interest to compare the productivity of the concen-

TABLE 2. *Dry-matter weight of 100 roots in 10^{-4} g.*

Colchicine concentration	NA concentration				
	0	11	7	5	Mean
250	439	842	815	595	673
200	988	700	640	938	817
150	765	1150	745	880	885
100	785	2104	763	825	1119
50	1200	1500	637	925	1066
0	1549	1627	1668	1480	1581
Mean	954	1321	878	941	1023

trations on both sides of the c-tumour threshold, in order to see whether the c-tumour corresponds in dry-matter content to a corresponding part of the normal root.

		NA			
		0	11	7	Average
Colchicine	100	785	2104	763	1217
	50	1200	1500	637	1112

No definite difference occurs; the few values at hand indicate that the grown-out root has about a corresponding dry-matter content to the tumour. Experiment 6 showed that the tumour corresponds to the normal elongation zone of the root. Counted from the root-tip the tumours and the elongation zone both extended within the second or (and) the third mm of the roots at the start of the experiment. Our experiments, thus, go to show that tumours developed under the influence of phytohormones, colchicine, many narcotics, *etc.* are the result of a change in the normally occurring longitudinal growth of the roots.

IV. INDUCTION OF LATERAL ROOTS.

The well-known effect of NA to start the origin of side-roots was observed in our experiments. In this respect the concentrations 4 and 5 turned out in all experiments to be most active. Already on the sixth or seventh day numerous small protuberances along the c-tumours appeared, and during the following days lateral roots broke through. As long as the treatment continues the side-roots behave like the main root, each of them forming tumours of their own. Even long after their transfer to water the side-roots remain unelongated. From concentration

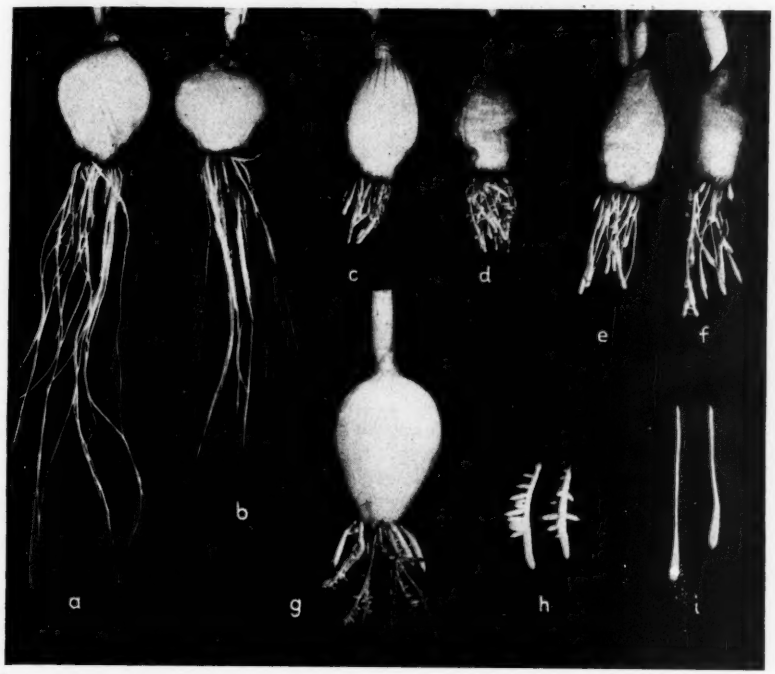

Fig. 6. *a—f*, *h—i*: bulbs of experiment 6 after 21 days of treatment, *g*: one bulb of experiment 4 after 11 days of treatment followed by 16 days in water. *a—b*: controls in water, *c—d*: NA 5, *e—f*: colchicine 200×10^{-6} mol/l, *g*: NA 5, *h—i*: details of the above bulbs *cd* and *ef*, respectively. — Photo H. OLSSON, Svalöf.

6 and downwards fewer lateral roots are formed. They appear later and grow out to normal side-roots. The controls sometimes form single side-roots, but usually no side-roots were formed during the experiment. Thus the control in experiment 2 gave some side-roots after 27 days. The controls of experiments 3, 4 and 6 formed no side-roots in 27, 22 and 21 days, respectively.

Treatment with colchicine alone has strangely enough in some cases permitted the initiation of lateral roots, this even in concentrations above the c-mitosis threshold. For instance, side-root formation was observed in 200×10^{-6} mol/l colchicine in experiment 6 after 21 days' treatment. The colchicine solution in this case was changed several times during the experiment, so there can be no question of any decrease in concentration of colchicine during the experiment due to

bacterial activity or some other similar cause. It was noticed that side-roots occurred only in roots where the primary meristem had ceased to show mitotic activity. As will be shown in the cytological analysis, these lateral root meristems, formed during a prolonged colchicine treatment, showed mitoses with normal spindle formation but often with a highly increased chromosome number.

In experiments 3 to 5 the effect of colchicine on the side-root formation induced by NA could be observed. It was found that the appearance of side-roots was retarded by colchicine but not completely checked. In experiment 4, for instance, small swellings appeared after 14 days' treatment. In the colchicine-free series of the same experiment there were already roots of 1 cm length at that time. Fig. 4 gives an idea of the development of side-roots in experiment 5. As seen, the lateral roots often start at the upper part of the tumours. Fig. 6 shows some instances of the appearance of side-roots in experiment 6.

V. CYTOLOGICAL EFFECT OF NA.

Generally speaking, the effects of phytohormones on root tissues and cells are rather well-known. Among histological effects the main one is the initiation of secondary root meristems along the pericycle. At the same time the cortical tissue swells up immediately above the tip meristem. By this process the tip meristem becomes more sharply delimited from the older differentiated tissues. Normally the transition between those two regions is very gradual and extends over a long region (about 1 cm). The sharp limit between meristematic and differentiated tissue brought about by strong concentrations (4 and 5) of NA is connected with the transversal swelling of the old cortical cells. In treated roots the lower limit of the old c-tumour corresponds to a rather precipitate change in cellular shape within the longitudinal cell-rows passing from the tip upwards. Just as the entire c-tumour corresponds to an extended region of the normal root, longer the older the tumour is, the transitional region between meristem and tumour in treated roots is condensed into a much shorter zone as compared with the normally occurring zone between tip meristem and elongation region of the untreated root. To the histological changes induced by concentrations 4 and 5 should also be assigned the change in orientation of mitoses in the tip meristem. As is well known, many mitoses are turned transversally instead of having the normal longitudinal orientation. This brings about a by-effect. The daughter cells of such divisions are

more in contact with each other than is the case normally when all divisions occur longitudinally. Instead of the normal, often very regular cell-rows the treated meristems show parcels of 4 or 8 cells, often resembling pollen tetrads.

Cytologically the NA has no very striking effect on the cells of the meristems, beyond the general toxic effects of strong concentrations. The most striking cytological effect is, however, the induction of mitoses in the old cortical tissue within the range of the c-tumour. In normal roots the corresponding cells do not enter mitosis at all, or do so only very exceptionally. Under the influence of NA in concentrations 4—6 these cortical cells begin after a few days to show mitoses. Our main interest was a study of these mitoses.

It has been shown on many occasions that these mitoses often contain the doubled chromosome number and that the doubling is produced endomitotically. As already mentioned in the introduction, it is not yet decided whether the phytohormone induces the chromosome doubling or only reveals the occurrence of it by starting mitoses. D'AMATO and AVANZI (1948), HUSKINS and STEINITZ (1948) and several others have given good circumstantial evidence for the second alternative.

In all our experiments we followed the cytological happenings in Navashin-fixed slides or in Carnoy-fixed Feulgen-squashes, according to the technique introduced by D'AMATO at our laboratory. Generally speaking, our results agree with those of earlier workers. Only in the course of the mitosis induced in old tissue have we some additions to make to what is already known, this mainly in what concerns interpretations.

Not even in the strongest concentration tested of NA (3), which concentration is near the solubility limit of the substance in water, was c-mitosis met with. Two hours' treatment in this concentration already showed clear toxic effects. After one day's treatment no more mitoses occurred in this treatment. The tissue was living, however, after two days in the treatment; longer treatment killed the roots. New roots grew out, however, if the bulbs were transferred to pure water. Treatment with this strong concentration often effected changes in the normal nuclear shape: angular or lobated nuclei were found together with other modifications in shape common in treatments with toxic concentrations of many substances. These types of nuclei after treatment with indole-acetic acid have been pictured, for instance, by DUHAMET (1945, p. 71, Fig. 5).

The concentrations 4—6 give the most striking cytological effects. Only two days' treatment start the transversal mitoses in the tip meristem, and mitoses begin to appear in the upper parts of the roots in the pericycle. Gradually a real meristematic tissue is formed around the vascular bundles. Soon, the mitotic activity of this tissue gathers to certain points outside the large vessels. These points later on give rise to lateral roots. While these changes are occurring in the interior of the root, the cortical parenchyma is also taking on its new appearance. There occurs no or very little growth in a longitudinal direction. Instead, the cells grow transversally, becoming more or less isodiametrical. The main cell layers responsible for this growth are the cortical layers. The epidermis and one or two layers below the epidermis do not grow transversally to any great extent. It is of interest that our comparative studies on tumours induced by NA and colchicine show a clear difference in organization of the two types of tumours. While the cortical cells after NA treatment still keep their normal arrangement as a mosaic pattern in which each cell is a polyhedron closely fitting into the adjoining cells and leaving little space for intercellulars, the colchicine-induced c-tumours show a cortical tissue in which each cell has grown out to a spherical bladder with thin walls, the intercellulars being considerable. Although we intend to look more closely into this condition, we may state already now that the cellular growth resulting from NA seems to be more regular, more normal, than that from colchicine. The coordination of growth is less disturbed in the former case. Measurements of cell-size have shown that the tumour cells of colchicine are of larger volume than those of normal cortical cells from the same region of the root, while those of NA are of the same size or sometimes even smaller. Especially in the younger parts of the colchicine-tumours each cell may have gone through c-mitosis and therefore in reality corresponds to two or more normal cells. So we are not yet able to decide whether the difference in shape of the cortical cells induced by NA and colchicine is due to the difference in cell volume. Taken together with other evidence mentioned above, *viz.* that the mechanism of development of NA-tumours may be different from that of colchicine-tumours, this distinction in organization of the cortical tissue in the two types of c-tumours is of some interest. The question will be studied further.

While the c-tumours of colchicine never contain mitoses, the NA-induced tumours often show mitotic activity in the old cortical cells. These abnormally occurring mitoses start after 3—4 days' treatment.

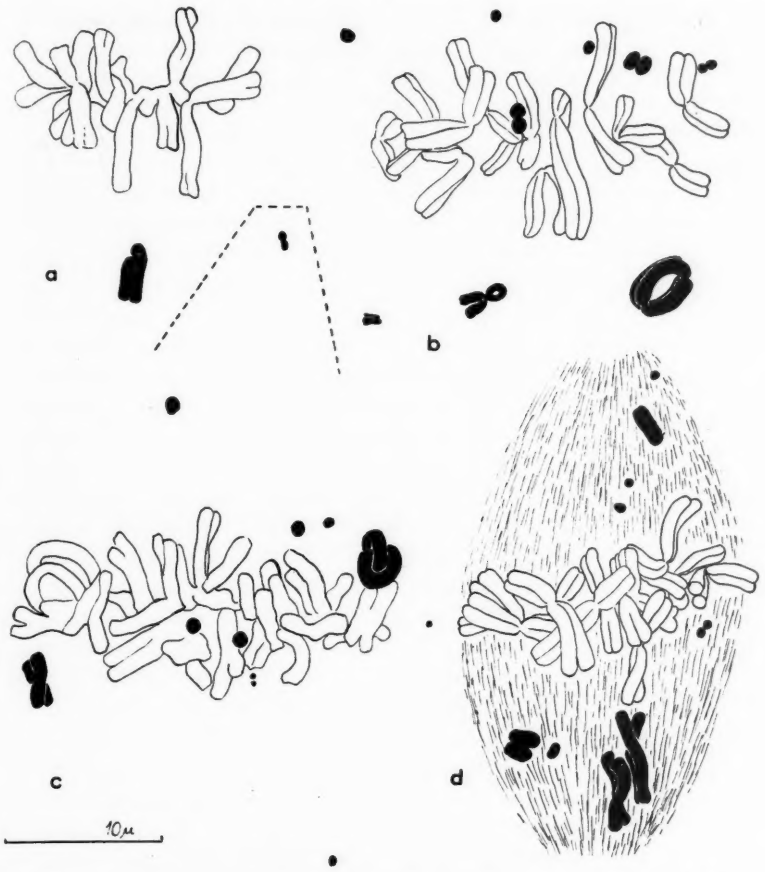

Fig. 7. Metaphases in (*a*) secondary root meristem, and (*b*—*d*) cortical parenchyma. *a*: NA 4, 4 days, *b*—*d*: NA 5, 7 days. — × 2400.

They are never very numerous; up to 50 mitoses may be found in one root tip, but normally only solitary ones are seen. Many of them are endomitotically reduplicated, but by no means all of them. The doubled ones increase in frequency towards older parts of the root. But even here often doubled and normal mitoses may occur intermingled in adjoining cells. The mechanism of induced endomitosis has been frequently studied (*e. g.* by DERMEN, 1941; BERGER and WITKUS, 1946; HUSKINS and STEINITZ, 1948; D'AMATO and AVANZI, 1948). Among the studies devoted to this type of mitoses D'AMATO's and AVANZI's (*l. c.*) is

TABLE 3. *Frequency of disturbed mitoses in tip meristem (1), lateral meristems (2) and cortical tissue (3).*

Region	1		2			3			
Root No.	Total of studied cells	Cells with bridges	Total of studied cells	Cells with bridges	Free or attached fragments	Total of studied cells	Cells with bridges	Free or attached fragments	Bridges and fragments
1	85	3	184	—	6	25	1	2	—
2	46	—	34	—	—	38	2	3	—
3	32	1	—	—	—	9	—	—	4
4	62	1	54	—	1	13	—	5	2
5	33	1	26	—	1	5	—	1	4
6	50	2	14	2	—	10	—	2	—
7	16	—	34	3	—	8	1	—	4
8	40	1	5	1	—	37	—	4	4
9	23	—	15	—	—	1	—	—	1
10	21	2	10	—	2	4	—	—	2
Total	408	11	376	6	10	150	4	17	21
% disturbed cells	2,70		4,26			28,00			

outstanding in the following respect. They record mitotic disturbances in anaphases and telophases of these divisions: »fenomeni di aberrazioni cromosomiche e disturbi anafasici più o meno intensi secondo i casi (veri ponti da fusione cromatidiale, frammenti semplici e doppi, cromosomi ritardatari 'ring'-cromosomi, nuclei sovrannumerari più o meno numerosi e di varia grandezza) ne sono la consequenza» (*l. c.*, p. 191).

Before knowing of these results we had come across abnormal anaphases and telophases in the cortical tissue of material treated with NA in the concentrations 4, 5 and sometimes 6. Our first thought was, naturally, that NA is another substance with radiomimetic effect, several of which have been found during the last few years (BAUCH, 1947; LEVAN and TJIO, 1948, *etc.*). However, we found that the disturbances occurred only in the old cortex, and not in the tip meristem or in the lateral meristems, although they had been equally exposed to the action of NA. In meristems there may be found occasional bridges caused by stickiness, but no real translocation bridges. As to fragments, we found several cases of attached fragments (*i. e.* induction of secondary constrictions) and one instance of a free fragment in secondary meristems (Fig. 7, *a*). The tip meristem has not been found to contain such disturbances.

In order to get an idea about the occurrence of the disturbances we divided the roots into three regions: (1) Tip meristem, (2) Meristems around the pericycle, and (3) Old cortex tissue. Within these regions we observed such anaphases as could be analysed, recording all cells with disturbances. The data are collected in Table 3. While the percentage of disturbances is very low in regions 1 and 2, it is considerable in 3. Our data show with strong evidence that this last region is much more liable to contain structural disturbances than are the meristems. There is also some indication of region 2 being somewhat more disturbed than region 1. At least the type of disturbances in region 2 is different, attached fragments not being rare. Within region 3 we did not record the various parts separately, but nevertheless it is quite apparent that the disturbances increase in frequency towards the upper part of the region, *i. e.* the part having the oldest cells. In some slides all cells in mitosis within this uppermost region showed disturbances. The percentage of disturbances, which in regions 1 and 2 is only 3—4, varies in region 3 from the youngest part to the oldest from a few per cent to almost 100 per cent. It was also noticed that almost all disturbances occurred in cells with doubled chromosome number. The undoubled cells often met with were usually normal, an observation also made by D'AMATO and AVANZI (*l. c.*). It can be established that NA as such does not produce the disturbances. It is clear that the condition of the cells at the treatment is of decisive significance.

The types of disturbances met with in these cortical cells are pictured in Figs. 7 and 8. Fig. 7, *a* is an exceptional cell from a secondary meristem and is one of the few cases where a real fragmentation occurred in a meristematic non-doubled cell. Fig. 7, *b*—*d* are mitoses from old cortical cells, all of which are endomitotically doubled, *c* possibly twice doubled (although hard to determine exactly; its chromosome number was closer to 64 than to 32). In these cells the great number of free fragments is striking. Evidently the fragments are symmetrical, for if only one chromatid is cut off, the fragment will usually keep its place in the chromosome until anaphase. The fragments are of all sizes from small minute ones to entire chromosome arms. It seems, however, that small fragments are in majority. Ring fragments are common, a ring with side-arms is seen in Fig. 7, *b*, two interlocked ring fragments in Fig. 7, *c*. Often the fragments are found far away from the equatorial plate in the outskirts of the spindle. At metaphase it is often difficult to analyse closely the position of the chromatids. Indications of translocations were often seen, however, as, for instance,

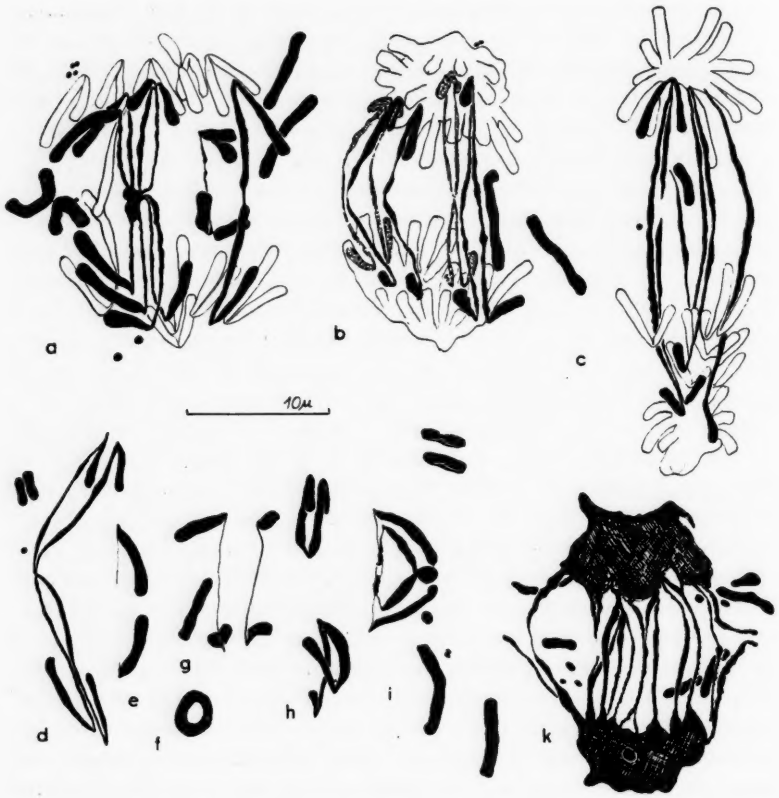

Fig. 8. Anaphases and telophase (*k*) in cortical parenchyma. *c*: NA 4, 4 days, the rest: NA 5, 7 days. *d—i*: structurally changed chromosomes from one cell, separately drawn. — × 2100.

to the left in the plate of Fig. 7, *c*, where two ring chromatids occurred.

At anaphase the structural abnormalities present are easier to detect and to analyse. Fig. 8 presents instances of such abnormalities from four anaphases and one telophase. They consist of bridges, loop chromatids (bridges within the anaphase group), fragments, *etc.* Even a superficial revision of the slides revealed one very striking feature. The bridges occur in pairs or often even in fours. In certain cases four bridges are directly involved in each other (Fig. 8, *a*), indicating a common development, in other cases they are free, but their position in-

dicates that they have been formed in pairs or in fours. This grouping of two or four together is so characteristic and so regularly occurring that it necessarily must be put in connection with the origin of these disturbances. It quite evidently has something to do with the phenomenon of endopolyploidy occurring in the cells. It is necessary to assume that the changes have occurred while the telophase chromosome derived from the last meristematic mitosis still behaved as a single thread. In that case the changes will be symmetric or double-symmetric. Double or quadruple bridges may be caused through one single change in the chromosome thread, if it reacts as a single thread, which from X-ray studies we know it does during the longest part of the resting stage. One break followed by sister chromatid reunion at this stage should give rise, after endomitosis, to two bridges and two fragments. A translocation between two chromosomes on the single stage should give rise to four dicentric chromosomes, which according to the orientation of the centromeres at anaphase should form 2 bridges + 2 loop chromatids, 4 bridges or, finally, 4 loop chromatids, these three types of changes occurring in the proportion 2 : 1 : 1 respectively. In this case the conditions may be further complicated by spiralizations occurring between the centromeres of the dicentric chromatids, which may effect interlockings between otherwise free bridges of loop chromatids.

According to this view upon the origin of the bridges, there should occur free fragments at least as numerous as the bridges. At first we found too few fragments at anaphase, but after having seen at metaphase many very small fragments, often at a considerable distance from the chromosomes, we were able almost regularly to detect also at anaphase the fragments belonging to the various bridges. In Fig. 8, *a*, *e. g.*, the two big fragments to the left probably belong to a loop configuration not represented in the picture, as it was concealed by the quadruple bridge. This latter configuration has, with great probability, its fragments in the upper part to the left on the picture, where four small fragments form a group. Although these fragments are regular in shape and clearly show their chromosomal nature, they exhibit some signs of destruction. It is possible that very small fragments gradually disappear, being digested in the plasm. In this case the translocation has evidently occurred close to the chromosome ends, which agrees well with the proportions of the chromosome arms in the quadruple bridge. The two big fragments up to the right on the picture probably belong to the second bridge formation counted from the right. This

consists of a double bridge, and the original break has occurred very close to the centromere, where both the centric and the acentric parts have undergone reunion. As a matter of fact both fragments showed in their middle a light band indicating the remnant of the old place of reunion.

Fig. 8, *b* shows very clearly two cases of four bridges. We only found two big fragments and two very small ones, the latter showing signs of destruction. Fig. 8, *d—i* represent, separately drawn, the various structural changes of one cell including bridges, loops, fragments, ring fragments, *etc.* Fig. 8, *k* is a telophase, where the changes have been very extreme, the cell showing indications of destruction.

It should be pointed out that the structural changes depicted from endopolyploid cortical cells by D'AMATO and AVANZI in their paper, cited several times, and induced by 6 days' treatment with 10^{-6} mol/l of another phytohormone (2,4-dichlorophenoxy acetic acid) were also of the same symmetrical type as found by us (*l. c.*, Fig. 12, *a*, *d*, *f*, p. 190). It is evidently a question of parallel formations. These mitoses induced by phytohormones contain disturbances of a similar kind to those described by GENTCHEFF and GUSTAFSSON (1939) after X-ray treatment of dormant seed in *Spinacia.* In the endomitotically reduplicated root cells, typical of this plant, the writers observed double and quadruple fragments, interpreted as breaks in the single chromosome thread followed by double reproduction of the fragments.

VI. CYTOLOGICAL EFFECT OF NA AND COLCHICINE.

In our experiments 3 to 5 the cytological effect of combined treatments with NA and colchicine were studied. In agreement with what was earlier found in connection with the c-tumour reaction, no pronounced effect of NA could be demonstrated on the position of the c-mitotic threshold of colchicine. In both experiment 3 and 4 the colchicine series showed c-mitosis all through the various NA concentrations, as also in the control colchicine and water. This behaviour was expected, since the colchicine concentration employed, 300×10^{-6} mol/l, lies well over the threshold. In experiment 5, on the other hand, the combined variation of both substances was studied. In this experiment one day's treatment gave the following reaction (+ = exclusively c-mitosis, ± = c-mitosis and normal mitosis, — = normal mitosis; the number after each sign is an estimation of the frequency of normal mitoses

according to a scale from 0 = no normal mitosis, to 10 = exclusively normal mitoses).

Concentrations of colchicine	Concentrations of NA 5	7	10	0
250	± 3	+ 0	+ 0	+ 0
200	± 5	± 4	(±) 0,5	± 7
150	± 7	± 3	± 2	± 5
100	± 9	± 3,5	± 6	± 8,5
50	(+) 9,5	(+) 9,5	(+) 9,5	— 10
0	— 10	— 10	— 10	— 10

Usually we mean by the threshold of c-mitosis the zone between the lowest concentration showing any signs of c-mitosis and the highest concentration with exclusively normal mitoses. As seen, the threshold value is not sharp in this concentration range of colchicine. In the water series it is rather well-defined, lying between 100 and 50. With what degree of probability this threshold value is representative in various experiments we dare not say. In the great number of experiments made at this laboratory with the use of the same onion material (a »Zittauer» stock) and with uniform technique we have found the c-mitosis threshold rather constantly in the neighbourhood of 100—150 $\times 10^{-6}$ mol/l.

According to the above table, NA has had no great influence on the threshold. Possibly the threshold has been a little lowered. In all three NA concentrations there were found certain c-mitotic tendencies in colchicine 50, but these tendencies were very weak. In NA 7 the lowering tendency is clearest; colchicine 100—200 gave decidedly fewer normal mitoses in this series than in the control. The strongest NA, concentration 5, had an evident effect in the opposite direction. There was here a quite clear frequency of normal mitoses in colchicine 250.

These results may be supplemented with those of experiment 4, where slides were analysed on five occasions. In spite of the use of a somewhat higher colchicine concentration in this experiment, *viz.* 300 $\times 10^{-6}$ mol/l, there was found in many cases a certain frequency of normal mitoses. Below, the significance of these mitoses and their distribution in the root will be discussed more closely. The results were as follows (all of the treatments were »+» according to the former estimation, the scale 0—10 is still the frequency of normal mitoses).

Days of treatment	Concentrations of NA									
	4	5	6	7	8	9	10	11	12	0
1	1	2	5	0	3	—	—	—	—	0
2	4	5	3	0	3	1	2	8	3	0
4	7	4	3	0	0	0	0	0	1	2
8	dead	3	0	0	0	3	3	0	dead	dead
12	»	0	0	0	0	0	dead	dead	»	»

It is seen that the NA concentrations of the strongest zone, the c-tumour zone 4—6, have a clearly raising effect on the threshold region, more normal mitoses occurring in these concentrations than can be due to mere coincidence, especially when seen in the light of the values of experiment 5 above. Further on, a centre of normal mitoses seems to be present in the zone of longitudinal growth stimulation, concentrations 10—12, but this centre is less pronounced and is less clearly distinguished from the controls, where normal mitoses also may be found in this colchicine concentration.

It should be mentioned that an increase in the proportion of normal mitoses by the addition of a chemical substance is more unexpected and points to a more specific mechanism than the contrary effect. We have found that most unspecific toxic actions lower the c-mitotic threshold of colchicine, probably because any toxic effect has a tendency to weaken the ability of the cell to develop a normal spindle. Thus, the use of distilled water instead of tap water or nutrition solution as a solvent for the colchicine effects an evident lowering of the threshold (LACZYNSKA, 1948). Such antagonistic effects to colchicine as that of sulfone amide (BAUCH, 1946) or that of ethanol (LEVAN and ÖSTERGREN, 1943) are therefore of a special interest. Especially the former instance is quite remarkable, the threshold of c-tumours is increased to 1000×10^{-6} mol/l and that of c-mitosis to 500×10^{-6} mol/l by an intermixture of 2 % *prontosil solubile*.

The incessant recurrence of normal mitoses in the colchicine treatments, and the development of lateral roots in NA solutions as well as sometimes in controls with colchicine concentrations above the c-mitotic threshold led us to make a close study of the conditions within the meristems. It is an old experience that, even after long colchicine treatment of a growth point, all cells do not become polyploid. After finished treatment there are usually found cells with the original chromosome number. These cells easily regain predominance within the meristem, a situation, well-known to polyploidy breeders, and which is usually

explained by the greater viability and more rapid division rate of the low-chromosome cells. During treatments with strong colchicine concentrations, *e. g.* 0,05 mol/l, normal divisions in the meristems are never met with. The presence of normal chromosome numbers after finished treatment is considered as due to the ability of certain cells to remain in the resting stage all through the period of treatment and thus avoid going through any c-mitosis. In the present experiments, however, the colchicine concentrations were low, only little above the threshold, and the occurrence of normal mitoses during the treatments was rather common.

On studying the location of the two types of mitosis within the root, the normal ones and the c-mitoses, we found that the c-mitoses were always heaped upwards in the root while the normal mitoses were to be found close to the tip. This distribution is well-known by all who have any experience of the *Allium* test. At the testing of threshold values in various c-mitotic substances normal anaphases are always looked for at the vegetative point itself. If no normal mitoses are present there, usually none are found in the older parts of the root. During long treatments this condition often results in a division of the root into two regions, one narrower apical portion of small meristematic cells where normal mitoses are found, and one broader upper portion of larger cells which go through c-mitosis and which gradually grow out in volume as their age takes them into that region which in the untreated root is the stretching zone (Fig. 9, *a*—*c*). As a result these cells are included in the c-tumour, which consequently from below gets a continuous addition of high-polyploid cells, each of which in reality corresponds to several normal cells. Within the oldest part of the c-tumour, on the other hand, each cell is only corresponding to one cell. In c-tumours induced by c-mitotic substances, therefore, often the largest cells are found in the lowest part, while in c-tumours produced by NA they are present in the uppermost part, as is also the case in untreated roots.

Sometimes the apical part of the root is of considerable size and divided into two zones, one lower with normal mitoses and one upper zone with c-mitoses, only above this zone does the c-tumour start. Such is the case if the normal mitotic activity is intense and if the spindles are oriented longitudinally. We found this situation in NA treatments within the zone of longitudinal growth stimulation. Fig. 9, *a* gives a picture of this condition. If the cells of the central vessel are used as a landmark, the normal mitoses occurred within the meristem from

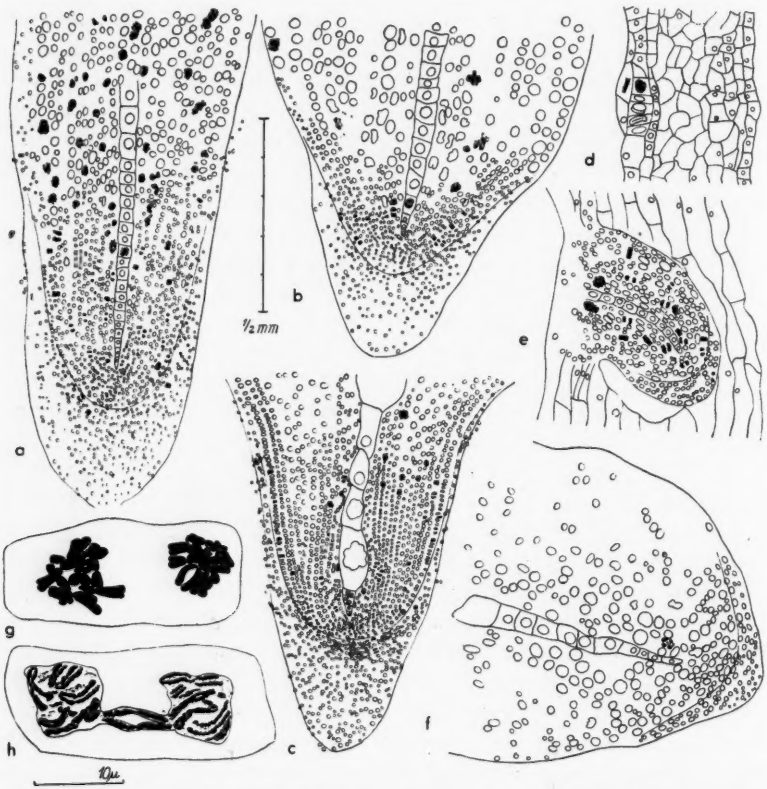

Fig. 9. *a*—*c*: primary root tips, *d*—*f*: lateral roots, *g*—*h*: distributive c-mitoses in tip meristem. *a*: NA 11 + colchicine 300, 2 days, *b*: NA 5 + colchicine 300, 8 days, *c*: NA 5 + colchicine 200, 15 days, *d*: NA 5 + colchicine 300, 8 days, *e*: NA 10 + colchicine 300, 12 days, *f*: colchicine 200, 24 days, *g*: NA 8 + colchicine 300, 1 day, *h*: NA 6 + colchicine 300, 4 days. — *a*—*f*, × 60, *g*—*h*, × 1400.

cell 1—13, above this zone there occurred exclusively c-mitoses within a root segment of about the same length. Above this the c-tumour began (not seen in the picture). In the transitional region between those two zones normal mitoses with tetraploid chromosome number were observed; one such anaphase is seen at the level of cell 13 of the central vessel to the left in the root. In this region c-mitosis and normal mitosis evidently alternate. The condition is labile, the least internal change may direct the mitosis in the one or the other direction. At the next testing of the same treatment (after 4 days' treatment) the roots still

showed the elongated meristematic zone, but in the roots tested most mitoses were c-mitoses, only solitary normals being found.

The occurrence of normal mitoses in the longitudinal direction, of course, brings about a length growth of the root. Since this type of mitotic activity was frequent in NA concentrations 10—12, undoubtedly these mitoses contribute to the observed increase in length in these concentrations (Fig. 3) even if the stimulation of the cellular stretching in the upper zone of the root is responsible for the main increase in length. That we do not obtain the same result in the higher concentrations of NA, although they still more evidently admit of the occurrence of normal mitoses, is due to the fact that these mitoses are often oriented transversally, just in the same manner as when colchicine is not present, and therefore add only to the broadening of the meristem.

Of a certain theoretical interest in our opinion is one observation made several times in these colchicine series. If mitoses occur in the central vessel, they are always c-mitoses even if they take place within that part of the meristem which contained normal mitoses (one case in Fig. 9, *a*). This condition gives a hint for the interpretation of the differential effect in the root of one and the same colchicine concentration. The cells of the central vessel are from the outset more differentiated than surrounding meristem cells. Far down in the undifferentiated meristem the central vessel may be recognized by larger cells and almost unstained nuclei, which only seldom and then in the lower part of the root undergo any mitoses. The nuclei are often found to be endomitotically doubled. They are in one word early-specialized, they lose their embryonic character early. Therefore it is highly interesting that, even if they are situated in the midst of a tissue undergoing normal mitosis, they still behave as the older, more specialized cells of the nearest older zone of the root. Our experiments show that the youngest, most embryonic cells keep their ability of giving normal mitosis in the presence of c-mitotic substances longer than any other more specialized cells. The youngest part of the meristem may be said to have a higher threshold value than the rest of the root. Here, all efforts of the roots are concentrated on keeping the machinery going. Even if all older cells have to be sacrificed, the meristem must continue to grow. If the external influence cannot be resisted, the meristem tries as a last resort to put off its activity: the important embryonic cells enter a resting stage and keep their positions until danger is over. We dare not say whether this resistance of the youngest embryonic cells to the colchicine action is of the same kind as the well-known resistance

to many kinds of infections shown by young children as well as seedlings.

Our analysis of the distribution of normal mitosis in the root meristem gives a somewhat different aspect to the returning of tissues to their original chromosome number after colchicine treatments. The ordinary conception of the treated tissue as a haphazard mosaic of $2x$, $4x \ldots n \times 2x$ cells is not exactly correct. The distribution of the various cell types is *not* haphazard, the $2x$ cells predominate in the very growth point and their starting-position after the end of the treatment is more favourable than that of their competitors. This, together with a more rapid mitotic rhythm, gives them good chances to recapture their predominance in the tissue.

After treatment with colchicine + strong NA concentrations it happens that certain cells of the central vessel become enormously enlarged and get giant nuclei. An instance is given in Fig. 9, *c*, where 15 days' treatment in NA 5 + 200×10^{-6} mol/l colchicine induced a very great increase in volume of the nuclei of the central vessel. The attaining of such a nuclear volume must have required a sequence of iterated c-mitoses. The largest nuclei in which the chromosome number after colchicine treatment has been directly determined have been 2^8-ploid or 256-ploid, which means a sequence of 7 c-mitoses in a row. D'AMATO and AVANZI (*l. c.*) have found very large nuclei in few-celled secondary root meristems after long colchicine treatment. In their case, evidently, a few pericycle cells have undergone c-mitoses which, if they had been normal, would have resulted in a multicellular root primordium. The largest nuclei in their material were 90—100 × 130—140 μ, and were visible to the naked eye. Our largest nucleus in the central vessel measured 64 × 86 μ, the number of c-mitoses for attaining this size may be estimated at 6 or 7. Since such large nuclei are not found in the central vessel after ordinary colchicine treatments, it is to be supposed that the NA starts a greater number of mitoses than are normal. As was already known to MANGENOT (1942), colchicine can be used as a means of estimating the number of mitoses which have occurred during the time of treatment. MANGENOT, using this criterion in combined treatments with colchicine and phytohormone, considers that the phytohormone (hetero-auxin) stimulates the number of mitoses. Although we have not made exact counts in our NA treatments, we have the impression, too, that most concentrations of NA increase the number of mitoses occurring in the meristem.

As already several times pointed out, lateral roots were frequent

in the NA treatments, even in the presence of colchicine. Pure colchicine treatments also gave solitary side-roots after long treatments, although usually not until the tip meristem had died. At the microscopic examination of these side-roots it was found that in the presence of 300×10^{-6} mol/l colchicine their development was often very slow and irregular. The mitoses of the pericycle were at first c-mitoses, which caused the chromosome number to be increased, sometimes quite extremely. Sometimes mitotic activity was seen in the pericycle, in which normal mitoses with high chromosome numbers were mixed with multipolar mitoses and pure c-mitoses. Often the starting root primordia were atrophying while they contained only a few large cells with high chromosome number (Fig. 9, *d*). In other cases the meristem was successful in starting normal mitoses, and then the side-root could become larger; often normal mitoses went on until the root broke through the cortex, forming a small side-root. In such roots very irregular chromosome conditions were found, mitoses with high numbers, 128 and 256, predominating in older parts of the root, while lower chromosome numbers occurred towards the tip (Fig. 9, *e*).

In the lower colchicine concentrations $250—100 \times 10^{-6}$ mol/l normal cells of the pericycle might occasionally directly give rise to side-roots without first having to go through any c-mitoses. These roots were, then, diploid from the beginning. Later it happened that c-mitoses started in the older parts of the side-root, while the meristem still grew on normally. They, thus, behaved just as the primary root. In such cases it could be seen that the tip meristem and the side-meristems contained normal mitoses, while older parts in both cases had c-mitoses. The embryonic cells, both of the main root and of the side-roots, kept resistant to the colchicine while physiologically older parts fell into c-mitosis.

VII. DEVIATIONS OF C-MITOSIS.

Our material, including a rich variety of treatments with colchicine in concentrations close to the c-mitotic threshold, gave a sample card of all those modifications of c-mitosis characteristic of such treatments. Commonest of these types is the so-called exploded c-mitosis, described by BARBER and CALLAN (1943) on newt mitosis. It is described by them in the following way: »... the chromosomes are pushed bodily to the boundary of the cell ... In the final condition the chromosomes may become segregated in several groups, but no separation of sister chromatids into two different nuclei takes place. ... The products of this

segregation are unbalanced and ultimately degenerate» (*l. c.*, pp. 262—263). A very common sub-type of these exploded c-mitoses is the one where the c-pairs are not scattered in all directions but only into two groups. This modification, which is mostly found in somewhat elongate cells, was described by NYBOM and KNUTSSON (1947) after treatment with methyl naphthoquinone under the designation »distributed c-mitosis». Superficially seen, this type of c-mitosis is strikingly similar to normal anaphase, especially if an entire meristem is filled with this type of c-mitosis, which sometimes occurs. It has been called pseudo-anaphase by some writers, but since it already originates during metaphase (*i. e.* before the division of the centromeres) we prefer the term »distributed» or, perhaps better, »*distributive* c-mitosis». It is characteristic of this mitosis that entire chromosomes are distributed into two groups within the cell (Fig. 9, *g*). Often single c-pairs are left lying between the two groups, strikingly resembling both in position and constitution lagging univalents in the first meiotic anaphase. After some time the centromeres divide, we get distributive c-anaphase and later c-telophase, when each group forms one nucleus, which is sometimes connected with the other nucleus by chromosomes left between the groups (Fig. 9, *h*). Cell-wall is not formed between the two nuclei, and if a new division follows it can be seen that the chromosome number of the whole cell has been doubled.

HUSKINS (1948, 1949 and elsewhere) has together with various collaborators described similar types of c-mitosis after treatment with sodium ribose nucleate in several plant materials, among them *Allium Cepa* roots. His excellent pictures indicate, according to our opinion, that it is a question of forms of c-mitosis mainly of the distributive type. He often uses the term »somatic meiosis» for this mechanism, a designation which we find inappropriate, since the resemblance to meiosis is only superficial. That the distribution of about half the number of c-pairs to each group is prevalent may be explained by the original position of the prophase nucleus within the cell. It is in meristematic tissue situated in the middle of the cell; at the »exploding» metaphase the c-pairs are spread out in all directions, which in oblong cells results in two groups of about the same size. We have difficulty in imagining any mechanism by which homologous c-pairs would be distributed to different groups, as is suggested by HUSKINS. Even if that be the case and cell-wall were formed between the groups, no numerical chromosome reduction would be effected, since the anaphase would restitute the original chromosome number in each cell.

Under such presumptions, it is true, the separation of homologous loci on two cells would be brought about. If, after that, these cells could go on dividing normally, there should occur twin isles of cells showing somatic segregation.

It should be pointed out that just in substances such as sodium ribose nucleate, which in strong concentrations give an incomplete c-mitosis (KIHLMAN and LEVAN, unpublished), all kinds of deviating types of c-mitosis and intermediate stages between c- and normal mitosis are frequent. Thus, HUSKINS' Fig. 1 E (1948, p. 310) corresponds to our type »bow-metaphase» (LEVAN, 1945), HUSKINS' Fig. 2 A (p. 317), which is designated »cell with two haploid nuclei», is an instance of two star metaphases in one cell (BARBER and CALLAN, *l. c.*). What HUSKINS refers to as »segregation and reduction» during prophase is in our opinion prophase in nuclei resulting from preceding exploded or distributive c-mitosis. KIHLMAN and LEVAN were not able to find, in their material, that a »second» normal division should follow upon the distributive c-mitosis with any regularity as HUSKINS seems to imply, for instance, in his Fig. 2 (1948, p. 317). As soon as the spindle mechanism is present, cell-walls are formed; therefore in cells containing multipolar anaphases in one process several small cells may be formed with varying chromosome numbers, often lower than the normal number of the species. Such cases have been described after all kinds of induced disturbances; very extreme ones were pictured in combined phytohormone and colchicine treatments *in vitro* of vascular parenchyma of the artichoke by MARTIN (1945).

The c-mitotic variations of which the distributive c-mitosis is a sub-type are thus not specific of any certain treatments but may be induced by most c-mitotic substances in concentrations close to the threshold. An especially great variation in type of c-mitosis is characteristic of the action of inorganic salt solutions (LEVAN, 1945). Even if one type of reaction predominates in a certain salt treatment it is hard to find any reactions specific of a certain substance. Therefore, the tentative correlation of disturbances induced by phosphates with any specific enzymic activity, as suggested by GALINSKY (1948), is according to our experience premature. All the many kinds of chromosome reactions found after phosphate treatments may be induced by several other inorganic salts, although, it is true, often with some little shifting in their relative frequency. Thus, the reactions of sodium phosphate buffer pictured by LEVAN (1949) in Fig. 2 (*l. c.*, p. 329) may be copied almost exactly by treatment with potassium cyanide, but in this

case there may be found partly a despiralization, which is never equally extreme in phosphate buffers, partly a c-mitosis, of the same completeness and type as in any organic c-mitotic substance, which is not to be induced, either, by the phosphate buffer.

Within that extremely fascinating range of cytology which the study of chromosomal responses to external influences constitutes there has now occurred an immense amount of more or less scattered observations. In our opinion, systematical observations on a standardized method are needed in order to attain a secure mapping out of the action of different types of substances, permitting comparisons of their effective zones, *etc.* This work is to a great extent routine and often tedious, but it will furnish a valuable basis for a real synthesis of the available data and for the further progress of this line of research.

VIII. THE CHROMOSOMAL DECAY OF AGING TISSUES.

In the present investigation we have twice met with the phenomenon that meristematic cells are different from more specialized tissues in type and behaviour of chromosomes. Under the influence of NA various kinds of structural disturbances appear in the specialized cortical cells, while the meristematic cells behave normally or have very much slighter disturbances. Under long colchicine treatments, especially such combined with NA, the meristems start to grow on and turn out to contain normal mitoses in the very growth point, while mitoses in older cells are of pronounced c-type. Although not comparable in detail, these two cases have one point in common, *viz.* that the meristematic cells are more resistant to injurious influences. The plant concentrates upon keeping its meristems to a normal chromosome behaviour.

The two instances are, as mentioned, not quite of the same kind: in the colchicine treatments it is probably the question of a certain acquired or inherent resistance to various unfavourable influences, as in this case to the colchicine, which is the inducing factor of the c-mitoses. In the NA treatments the situation may be that the structural changes already present in the old tissue are only revealed by the NA. That cells of increasing age contract damages in their chromosomes, is a condition already long known from other types of experiments. In aging seed, where the meristematic cells for years are kept in the deepest resting stage, there appears at the germination a greater frequency of structural abnormalities than in fresh seed. There is an in-

vestigation on this condition in *Allium Cepa* (NICHOLS, 1941). Germinating seed of different varieties exhibited a surprisingly high percentage of structural disturbances, often about 2 % of the analysed cells had structural abnormalities, and in one variety as much as 13 %. These frequencies increased considerably in stored seed. On the other hand, no such disturbances were found by NICHOLS in roots grown out from bulbs (in this case only relatively fresh material was studied). This condition was put in relation to the heavier dehydration of meristems in seed as compared with those in bulbs. The dehydration was considered to further the origin of structural changes. In a later paper (NICHOLS, 1942) this situation is further illustrated. For the further growth of the meristem the abnormalities found seemed to be of little significance. The disturbed cells soon disappeared, just as a high frequency of disturbances after X-radiation usually disappears in a short time, if only the meristems grow on at all.

In our case no serious disturbances after NA treatment are found in the chromosomes of meristematic cells. The cortical cells, as sometimes also the vascular cells, show numerous disturbances, more numerous the higher up in the root the cells are situated, that is the older they are. These cells belong to a tissue which normally will never show any mitoses, or which at least will never form any meristems or take part in the germ track. In untreated roots it is found that the most characteristic feature of these cells is their longitudinal growth; they are many times the volume of the meristematic cells. Often the cells have a large central vacuole, leaving around the walls a thin layer of plasm in which the nucleus is embedded at one place. We have made numerous measurements of nuclei in untreated material in order to get some information about the behaviour of these nuclei in respect of endoduplication. In the meristem the resting nuclei have a rather equal size and a spherical shape. In the old cortical tissue the nuclear size is variable, and the shape is spherical or lentiform. Our measurements from different regions within the differentiated part of the root gave the following results. Immediately above the meristem the nuclear size was rather uniform, somewhat larger than in the meristem. Higher up in the cortex it was highly variable, some nuclei being much larger than those of the meristem, some being also decidedly smaller. In their organization these old resting nuclei are characteristic. The chromosome matter is dispersed into very fine grains, though the prochromosomes are often visible. Nucleoli are small and stainless or even impossible to detect. We came to the conclusion that the cortical cells in spon-

taneous material did not behave uniformly, some probably did not undergo endomitosis but remained undoubled until their death. Others, which often are double the size or more of the meristematic nuclei, and in which the prochromosomes are about double the normal number, probably represent spontaneous endoduplications. In the extremely rare spontaneous divisions of this tissue it has sometimes been possible directly to observe the higher chromosome number and the characteristic diplochromosomes. In the central vessel such endomitoses are not seldom met with, even in its lower part. But normal diploid divisions are found now and then in the cortex too, as well as in the central vessel.

Our measurements of nuclei in NA induced c-tumours after 7 days' treatment showed that the number of large nuclei in the cortex was considerably increased. Whether this is a direct effect of the NA cannot be decided; it may be connected with the rather profound histological changes in the growing out of the c-tumour. The starting of mitoses, however, is due to the treatment, although even here it cannot, of course, be stated that the effect is a direct one on the cortical cells. It was often seen that the cortical mitoses occurred right outside those points of the pericycle where mitotic activity had started. If the phytohormone starts the embryonic mitoses in the pericycle, it may be that these, secondarily — for instance, through some mitotic substance diffusing out into the surrounding cortex — start the mitoses here. The cortical mitoses may be sometimes normal, or usually tetraploid and in solitary cases octoploid (twice endoduplicated).

It was shown in the present study that the mitoses of the cortex had the peculiarity of containing chromosomal aberrations, often of a nature serious enough to cause a complete break-down of the mitosis. The type of changes in the chromosome structure observed in these reduplicated nuclei show that in most cases the changes had been happening while the chromosome thread was functioning as single. Here may be found circumstantial evidence that the chromosome reduplication, in some cases at least, occurred after the induction of the disturbances. If all the structural changes found do not take place immediately after the end of the last meristematic telophase, but happen now and then during the long subsequent resting stage, it must be supposed that the chromosomes keep single during this stage and that the occurring doublings come about in connection with the unexpected onset of the prophase, directly or indirectly started by the NA.

During the collecting of these data we were brought along to a

working hypothesis about the chromosomal decay of old, specialized tissues. When a cell has gone through its last mitosis and thereafter is specialized in one or other direction, into a parenchyma cell, a vascular cell, *etc.*, its nucleus enters a final resting stage which will not be broken off by any mitoses but which sooner or later leads to the natural death of the cell. We know that in certain specialized tissues, *e. g.* the salivary glands and the iliac epithelium of Diptera, the chromosome apparatus is kept in perfect order during the iterated endomitoses occurring. In other cases, as in the root cortex, it seems that the chromosomes have played their rôle to an end. The cell does not need to keep up its chromosomal mechanism, the valuable accessories involved herein may be spent on other tissues, more in need of mitoses. Our pictures (Figs. 7 and 8) show remarkably often small paired fragments. These undoubtedly correspond to the chromosome ends. These »telomeres» have the function of protecting the ends of the genic thread from fusing with each other and in that way causing trouble at the next mitosis. If no more mitoses are to follow in a cell, the telomeres become utterly unnecessary. Their elimination has the consequence that two chromosome ends that happen to come in contact will fuse, which is of little consequence in a definitively resting nucleus. According to our point of view, the ability of accomplishing a normal mitosis may become lost within a tissue which does not need to keep up this ability.

If later, due to unforeseen circumstances, a mitosis gets started, it may be through the supply of a phytohormone or through a beginning cancer growth, it is made evident that the ability of a normal mitotic course is lost. In cancer tissue chromosome disturbances of structural type are common. It is possible that in some cases they are of similar origin to the disturbances in our old cortical cells, *i. e.* they may be due to chromosomal decay by age.

The physiological age of a tissue is a relative conception, which must be seen in connection with the tissue's length of life and its degree of differentiation. Only the germ track is of eternal youth. From the standpoint of the species the cells of the germ track are the only really important ones. These cells are most resistant to various external influences; they are the first ones to recover after poisonings. When a tissue specializes, this happens at the cost of some of its original power of resistance. However, the specialization need not mean that the cells definitively lose their meristematic character. In many cases a part of a leaf, or of a rhizome, may give rise to entirely new individuals. Certain of the cells in the specialized tissue have re-entered the germ track. In

other cases, as for instance after an endomitosis, the exit from the germ track should be more final. But cases are known where callous cells treated with phytohormones and supposedly endoduplicated may give rise to chromosome-doubled shoots, as in the well-known *Nicotiana* experiments by GREENLEAF (1938). The case is different, however, with our cortex cells in *Allium* roots. In these cells it is favourable neither for the species nor for the individual that the meristematic ability is maintained.

It may be possible that our point of view stresses a little too much the indifference of the condition whether the genes of a resting nucleus are organized into chromosomes or not within an aging tissue. It may of course be of much different importance in different tissues. If, for instance, a genic position effect should be of serious influence for the normal functioning of the tissue in question, the chromosomes have to be protected against the occurrence of structural changes. Maybe the changes of the chromosomes, which in our view must be regarded as a decay, only occur in tissues so close to their death that the changes are really to be looked upon as premortal atrophying processes. In that case it must be considered as interesting that the supply of phytohormone can check this process of atrophying — it may be only for a short time.

SUMMARY.

The present paper reports some experiments on the influence of naphthalene acetic acid (NA) alone and in combination with colchicine on root meristems of *Allium Cepa*. A concentration series of NA from 10^{-12} to 10^{-3} mol/l (0,000·000·2—186,2 ppm) and colchicine concentrations around the threshold concentration of c-tumours and c-mitosis, 50—300×10^{-6} mol/l (0,002—0,012 %), were tested.

NA alone gave growth inhibition of the roots in the strongest concentrations tested, 3—6 (designated according to the negative exponents of 10), and growth promotion in the weakest ones, 11—12. Tumours on the roots (c-tumours) were present in NA 4—6 and in colchicine 100—300. Some evidence was collected that the c-tumours of NA are of a somewhat different nature from those of colchicine.

C-mitosis was induced by no concentration of NA, but by the concentrations 100—300 of colchicine. In combined treatments it was shown that the growth-promoting concentrations of NA in some degree counteracted the growth inhibition of colchicine. The cytological study revealed a higher number of normal mitoses in the roots treated with

colchicine and NA than in those treated with colchicine alone. In the higher concentrations of NA these normal mitoses were oriented transversally in the meristem and, therefore, did not add to the longitudinal growth of the roots, but in NA 11 and 12 + colchicine many normal mitoses were present directed longitudinally and thus effected an increase in root length. It was demonstrated that the normal mitoses, occurring in colchicine concentrations above the threshold value of c-mitosis, were often located in the very point of growth, while older parts of the roots contained exclusively c-mitosis. The same situation was present in the lateral roots, which were found to develop even in concentrations above the c-mitotic threshold. The youngest and most embryonic tissue, therefore, seems to be more resistant to colchicine than the more specialized tissue.

The mitoses, now and then started by NA in the old cortex tissue, were closely studied. As was earlier known, their chromosomes are often endomitotically doubled. It was found that these mitoses often contain structural disturbances, which were of an interesting type, indicating that usually they had originated while the chromosome thread reacted as single after the last meristematic telophase. Since no or very few such disturbances were found in the meristematic cells of the same root tips, it was concluded that the NA *per se* does not induce the disturbances. NA only acts as a detector of structural changes which are probably inherent in the old cortex cells, and which would normally be of no consequence for the tissue and never become observed. The phenomenon of chromosomal decay from aging is discussed.

Various deviations from the typical c-mitotic course are described, and the »somatic meiosis» of the HUSKINS school is examined somewhat critically.

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SUPERNUMERARY CHROMOSOMES IN GODETIA VIMINEA

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THE genus *Godetia* has rather different chromosome numbers. In the species cultivated by Dr. GUNNAR HIORTH (HÅKANSSON, 1941; HIORTH, 1941) which probably constitute only a fraction of the total, there were the haploid numbers 7 (*G. amoena, Whitneyi, hispidula*), 14 (*G. nutans*), 8 (*G. biloba*), 9 (*G. deflexa, Bottae, cylindrica, parviflora, viminea*), 17 (a South-American species-group), and 26 (*G. purpurea, quadrivulnera*). In nature there are »monosomics» of *G. Whitneyi* with $2n = 13$, and as a result of crosses between different monosomatic types a *G. Whitneyi* with $n = 6$ has arisen (HÅKANSSON, 1946). One or two races of *G. nutans* had »oscillating» chromosome numbers, *e. g.* plants from Santa Rosa had 28, 29 or 30 chromosomes and the offspring of self-fertilized 30-chromosomal plants showed 28—32 chromosomes (HÅKANSSON, 1945). The supernumerary *nutans* chromosomes, however, showed a peculiar pairing and had no or little effect on the plant's morphology. For these reasons they were interpreted as accessory chromosomes or B chromosomes, although they did not resemble B chromosomes in other respects, having the same size as the A chromosomes and not being heterochromatic.

At the cytological examination of *G. viminea* only the meiosis of two plants was studied. During metaphase 1 one of them showed 9 II + 1 I and the other 9 II + 2 I. Concerning the origin of the *viminea* plants examined HIORTH has stated (1941, p. 207): »Samenprobe ursprünglich von Lester Rowntree. Da diese Form einer gleichzeitig ausgesähten, von Dr. D. A. JOHANSEN empfangenen Probe von *viminea* sehr ähnlich war, dürfte es sich hier zweifellos um die Art *G. viminea* handeln.» However, the univalent chromosomes were not fragment chromosomes, but of the same order of size as the other chromosomes, and therefore it appeared to me probable that the plants examined were derived from some species-cross. Thus, only the bivalent-forming chromosomes were assumed to be *viminea* chromosomes, the univalents were foreign to the species. At first there may have taken place a cross between *G. viminea* and some *Godetia* species with a higher chromo-

some number, and after that backcrosses with *viminea*, though with some of the chromosomes belonging to the foreign species still left in the population. Still, the observation of accessory chromosomes of the same size as the normal chromosomes of *G. nutans* had to have its influence on the interpretation of the nature of the supernumerary *viminea* chromosomes. It no longer appeared so certain that it was a question of species-foreign chromosomes in the usual sense of the term. Fresh investigations were therefore of interest. On a couple of occasions (1943 and 1947) I have also received from Dr. HIORTH seed specimens of the same Californian material as was previously studied.

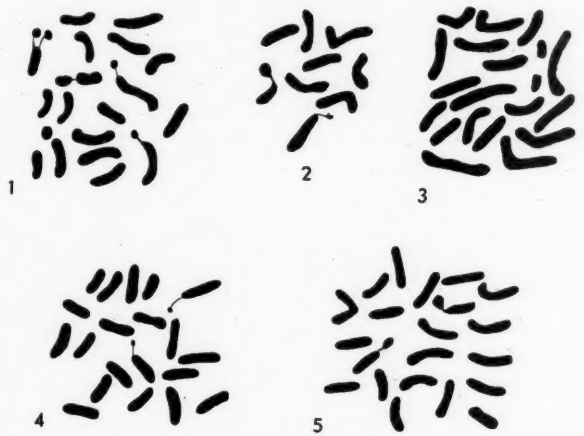
All the seeds were from plants that had been self-fertilized in 1940. The chromosome number of the mother-plants is unknown. Seeds from one of the plants were sown out at Svalöf in 1943 as well as in 1947, from the others in 1947. The seeds sown in 1947 must be regarded as rather old, which is presumably the reason that the germination was poor in a few cases. A number of the young plants were deficient in chlorophyll, but this had nothing to do with the presence or absence of supernumerary chromosomes and nor did it seem to have any connection with the age of the seeds, since there were defective plants in both 1943 and 1947. Only one progeny had supernumerary chromosomes (its mother-plant is here designated plant 5), the others consisted exclusively of 18-chromosome plants. One progeny, however, consisted of only three plants and in this case it is therefore uncertain whether the maternal plant had really been devoid of supernumerary chromosomes. On the other hand, in three progenies 18 chromosomes could be counted in the root-tips of 40, 20 and 17 plants, respectively, and in these cases it is highly improbable that the mother-plant had possessed another chromosome number than 18. Hence, in HIORTH's first material there were plants with and without supernumerary chromosomes.

MITOSIS.

The chromosomes are small and mostly there was no constriction at the spindle attachment. It was therefore not possible to make a close morphological analysis of the 18-chromosomal plate (Fig. 1; *cf.* the haploid plate in Fig. 2). It could be established that the chromosomes had different sizes but that there was a gradual transition from the largest to the smallest. Observations of anaphase show that several chromosomes have an almost terminal spindle attachment, that others have arms of unequal length and that only few have a submedian

attachment. The plate is rather like that of *G. deflexa*, though the chromosomes of the latter species are considerably larger. Some of the chromosomes bear small satellites. However, not more than three such typical SAT chromosomes have been observed, whereas there ought to have been four. In the majority of cases only two can be discovered, and this suggests that certain satellites are visible only when the fixation is very good.

Finally, a very characteristic feature of *G. viminea* consists of two small chromosomes with very large satellitic appendages. Often these



Godetia viminea, root tip mitosis in *1*: normal plant. — *2*: haploid plant. — *3*: plant with fragments. — *4*: plant with two supernumerary chromosomes. — *5*: plant with three supernumeraries.

appendages looked like free fragments because the thread uniting the appendage to the chromosome itself was unstained and, in addition, could be rather long.

Real fragment-chromosomes were nevertheless observed. This was in the offspring which had been sown in both 1943 and 1947. Some of the plants fixed in 1943 showed at root-mitosis two small fragments (Fig. 3 shows a plate lacking visible satellites) in addition to the 18 normal chromosomes. In some plates only one fragment could be seen, and in many none at all. Efforts to discover the fragments at meiosis were unsuccessful. It therefore seems as though the fragments are easily lost during the divisions.

Some plants had been subjected to cold treatment (for 16 hours at

0° C.), the object being to ascertain whether after cold treatment special segments became visible in the chromosomes. No such segments could be discovered, however. Divisions were rare in the cold-treated roots. Prophase nuclei had a diakinesis appearance on account of the chromosomes being short, thick and divided. Only few metaphases were observed; on the other hand, there were numerous binucleate cells. Proceeding nuclear divisions had thus terminated but cell-formation had been prevented and the frequency of new divisions had been greatly reduced.

Among the progeny of plant 5 it was possible to examine 18 plants. Only three had 18 chromosomes, fifteen had supernumerary chromosomes. Nine plants had 19 chromosomes, three had 20 and three had 21. Hence plant 5 had possessed supernumerary chromosomes. The small number of 18-chromosomal plants indicates that plant 5 had had more than one supernumerary chromosome (*vide infra*). This cannot be considered as certain, however, for the seed was old and therefore the possibility of a selection must be taken into account: embryos with supernumerary chromosomes may have a longer length of life. The number of supernumerary chromosomes was thus 1—3. They were not so unlike the normal chromosomes that they could be recognized on the plates. Observations of meiosis indicate that they have an approximately median spindle attachment, but this did not make recognition easier on the root plates, as the position of the spindle attachments was indistinct.

The progeny of some of these plants were sown in 1948. Flowers had been isolated and then self-pollinated. The largest material was obtained from plant 5—1, which had 19 chromosomes. Roots of 55 plants in the progeny had been fixed and the chromosome number could be determined in 52 of these. From some of them flower-buds had also been fixed. One plant had 9 chromosomes, nineteen had 18, twenty had 19, nine had 20 and three plants had 21 (Figs. 2, 4, 5). In some cases a certain variation was found in the chromosome number within the same root. A plant in which, as a rule, the plates had 19 chromosomes had a couple of plates with 20. Further, two 20-chromosome plants presented some plates with 21 chromosomes. This variation is presumably due to rarely occurring disturbances of the root-mitoses. In one 18-chromosomal plant a plate was observed with 19 chromosomes in the epidermis. Judging from the appearance of the plate, one chromosome had undergone premature division in this last-mentioned case. The result of this self-fertilization shows that plant 5—1 cannot

have been an ordinary trisomic. Had this been the case, the progeny would have consisted only of plants with 18 and 19 chromosomes, whereas in this case twelve plants (about 25 %) had a higher number. On the other hand, if it is assumed that the supernumerary chromosome does not undergo any elimination at meiosis and that embryo-sacs, pollen-grains or embryos with supernumerary chromosomes have no weakened vitality, 25 % 18-chromosomal plants should have been formed: 50 % 19-chromosomal: 25 % 20-chromosomal plants. As against this the progeny obtained had too many 18-chromosomal and too few 19-chromosomal plants. Further, there were some plants with unexpected chromosome numbers. Of interest is the appearance of a haploid, which had thus arisen after self-pollination and doubtless by parthenogenesis; five roots examined all had 9 chromosomes (Fig. 2), but unfortunately meiosis could not be studied. Three plants had 21 chromosomes, *i. e.* too many, for this number cannot be accounted for by the distribution of the supernumerary chromosome during meiosis.

Another self-pollinated plant, 5—7, also had 19 chromosomes. Unfortunately the progeny was small and only 10 plants could be studied. Four of these had 18 chromosomes, six had supernumeraries. The latter plants had very indistinct plates. Only one plant had 19 chromosomes, two had 20 (observed at meiosis) and one had 21 (also observed at meiosis). In the case of two plants, finally, it was not possible to decide whether the chromosome number was 20 or 21. It is regrettable that a larger progeny was not obtained from this plant, as there accordingly exists an intimation that the two self-fertilizations had given different results.

MEIOSIS.

The study of meiosis is more troublesome than in other *Godetia* species. As in other species, the loculi of the anthers are divided into compartments, and in each of these the P.M.C's are in about the same stage of meiosis, but in *G. viminea* the compartments are very small, which renders it more difficult to make a large number of observations. Moreover, in many compartments the P.M.C's are badly fixed. In other respects, too, *G. viminea* seems to be rather an unfavourable material for studies of meiosis.

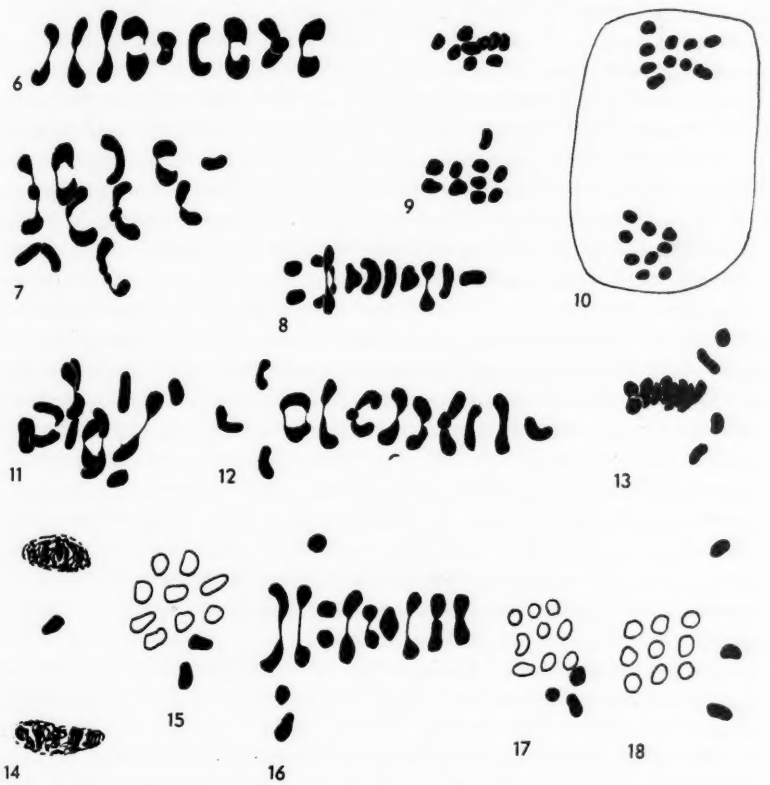
Meiosis of 18-chromosome plants. — Seven plants fixed in 1943 and one fixed in 1948 were studied. The chromosomes formed 9 II and the divisions were regular (Fig. 6). There was however a weak tendency to asyndesis, a number of P.M.C's having 8 II + 2 I. This asyndesis

was most pronounced in the plant fixed in 1948. Here, four univalents could be observed now and then. The asyndesis may have been due to environmental factors. During anaphase the univalents behave in different manners. They may undergo division or may pass undivided to the pole.

Meiosis of 19-chromosome plants. — Four plants could be studied. As a rule the supernumerary chromosome was unpaired, the 1st metaphase thus showing 9 II + 1 I. Fig. 7 is a pro-metaphase with unusually scattered chromosomes. Fig. 8 shows the chromosomes from metaphase 1 with the univalent on the equatorial plate. In most of the P.M.C's, as a matter of fact, the univalent lay in or among the bivalents. In many cases, as it seemed, its spindle attachment was exactly in the equatorial plane of the spindle, in other cases the univalent lay somewhat above or below this. Sometimes, however, it was at a greater distance from the plate and in one of the plants this was the case in the majority of P.M.C's. In well-fixed flower-buds the univalent was often rather long and appeared to have a median spindle attachment, although it was often short and almost isodiametric. At times no univalent could be observed, this doubtless being due to its being concealed by the bivalents on the plate. No undoubted example of trivalent-formation was observed; hence the supernumerary chromosome does not seem able to pair with any of the ordinary chromosomes. This argues strongly against the 19-chromosomal plants being trisomics.

In spite of the fact that the univalent is on or near the plate it undergoes no division during anaphase 1 but passes undivided to the pole together with one of the anaphase groups. It may be somewhat delayed (Fig. 9), though seldom so much that it is not included in the telophase nucleus. Its behaviour may be compared with that of the univalent in monosomics of *G. Whitneyi*. In the latter species, too, the univalent has its position on the plate and undergoes no division, being, however, left at the equator or passing much belated to the pole. The univalent shows similar behaviour in certain *Whitneyi* plants with a supernumerary *deflexa* chromosome. The result is that the univalent chromosome very often becomes eliminated in these cases. In *G. viminea*, on the other hand, the univalent may certainly be eliminated (*cf.* Fig. 14), but this only takes place relatively seldom.

P.M.C's with 8 II + 3 I can be observed. Hence a certain amount of asyndesis occurs, as in the normally chromosomed plants. This asyndesis explains why once during second metaphase 11 chromosomes were observed on one metaphase plate and 8 on the other: three uni-



Godetia viminea, meiosis. — *6*: the bivalents of a normal plant. — Figs. 7—10: plant with one supernumerary. *7*: pro-metaphase, chromosomes *in situ*. — *8*: the chromosomes at the first metaphase. — *9*: the first anaphase. — *10*: the second metaphase. — Figs. 11—15, plant with two supernumeraries. *11*: pro-metaphase. — *12*: the chromosomes at the first metaphase. — *13*: the first metaphase with 8 II + 4 I. — *14*: the first telophase, one chromosome eliminated. — *15*: the first metaphase 9 II + 2 I. — Figs. 16—18, plant with three supernumeraries. *16*: the chromosomes at the first metaphase. — *17—18*: the first metaphase 9 II + 3 I.

valents had passed to the same pole. Usually the one plate has 10 and the other 9 chromosomes (Fig. 10). Disturbances of the second division are rare. This was to be expected, since as a rule there are only undivided chromosomes after anaphase 1.

Meiosis of 20-chromosome plants. — Four plants were studied. In all of them the two supernumerary chromosomes were unpaired, there

thus being formed 9 II + 2 I. Pro-metaphase is illustrated in Fig. 11. During metaphase 1 both the univalents are often arranged on the equatorial plate (Fig. 12) or are in its vicinity. But often one univalent or more rarely both univalents are seen at a longish distance from the plate. Rather frequently the univalents are near each other as though there were an attraction between them (Fig. 15). MÜNTZING has observed that the B bivalents in *Poa alpina* are often in contact at diakinesis, which he presumes to be dependent upon heterochromatin. At metaphase 1 there is no contact between these B bivalents, but as a consequence of their earlier union they are often near each other. Something like this might be true of the supernumerary chromosomes of *G. viminea* (the diakinesis stages in this species were unsuitable for close studies). As regards *G. viminea* an explanation without recourse to the assumption of heterochromatin is also conceivable, *viz.* that the univalents may often be paired normally during meiotic prophase and the partnership be dissolved rather late. The question is, however, whether this can account for the occurrence of univalents lying alongside each other at the same side of and at a distance from the plate; rather they ought to have come to lie each on its side of the plate. During anaphase and the second division the univalents behave in the same way as in the 19-chromosome plants. Only more rarely does elimination take place.

In the 20-chromosome plants, too, P.M.C's with asyndesis among the normal chromosomes are found. The pairing 8 II + 4 I is in reality rather often found (Fig. 13).

Meiosis of 21-chromosome plants. — Only one plant, 5—7—6, was studied. Fig. 16 illustrates the chromosomes from a metaphase 1. The three supernumerary chromosomes are unpaired and in this case do not lie on the equatorial plate. The normal pairing is 9 II + 3 I. Fig. 18 shows a transversely cut metaphase in which the three supernumerary chromosomes form a group of their own. In Fig. 17 there is a longer distance between the univalents.

Elimination of supernumerary chromosomes is not common in the 21-chromosomal plant either. Asyndesis among the normal chromosomes can be observed rather often. 8 II + 5 I was observed, but the material was too small for close studies.

The variable appearance of the univalent supernumerary was particularly striking in this plant with three such chromosomes. Clear evidence that isodiametric univalents were ring univalents could, however, not be obtained.

CONCLUSIONS.

New investigations of *Godetia viminea* have confirmed that there are supernumerary chromosomes in this species but have also shown that such do not occur in all plants. The material studied consisted of some progenies of self-fertilized plants, and it is not possible to form any opinion as to the frequency of plants with supernumerary chromosomes in nature. Progenies of plants having supernumerary chromosomes, which have been self-fertilized, have proved to consist of (1) plants with 1, 2 or 3 supernumerary chromosomes, (2) plants with the normal number of chromosomes (18). The latter were many, $^1/_3$—$^1/_2$ of the total number, but a series of facts argue against the supernumerary chromosomes being ordinary *viminea* chromosomes that had been doubled. The 19-chromosome plants cannot be ordinary trisomics; in that case trisomics would be involved that give rise to a larger number of tetrasomics and even pentasomics, which do not exhibit any phenotypic peculiarities but look like ordinary *viminea* plants. This seems unreasonable. The observations made of meiosis also argue against trisomia or polysomia.

The view advanced in 1941, however, *viz.* that the *viminea* plants then studied were of hybrid nature, containing chromosomes foreign to the species and unpaired at meiosis, has hardly received any support from the new investigations. In the progeny of a 19-chromosome plant the 20-chromosome plants have two identically similar supernumerary chromosomes and the 21-chromosome plants three such. At meiosis 10 II should have been found in the former case and 9 II + 1 III in the latter case, but strangely enough the supernumerary chromosomes in both the plant-types were unpaired. It seems as though the supernumerary *viminea* chromosomes cannot pair either with the normal chromosomes or reciprocally. This peculiarity, together with the fact that the supernumerary chromosomes appear to exercise but little effect in a morphological respect, impels us to consider whether the supernumerary *viminea* chromosomes are not new specimens of the category denoted as »accessory chromosomes». Such chromosomes have in recent times been the object of detailed studies, especially in rye (above all by MÜNTZING) and in maize (RANDOLPH and other workers). In several other objects supernumerary chromosomes have also been found to have remarkable properties that have caused them to be designated B chromosomes or accessory chromosomes. Certain peculiarities are concerned that seem to be especially characteristic of accessory chromo-

somes (HÅKANSSON, 1945), and the question is to what extent the *viminea* chromosomes possess such properties.

As a rule B chromosomes are smaller than the ordinary chromosomes (the A chromosomes). The supernumerary *viminea* chromosomes, however, are of normal size. Examples of large B chromosomes are nevertheless known, *e. g.* one of the three B types in *Sorghum purpureosericeum* (DARLINGTON and THOMAS, 1941), the so-called large isofragment in *Secale* (MÜNTZING, 1944). The accessory chromosomes in *Godetia nutans* had normal size. In the two first-mentioned cases B chromosomes often appear during metaphase 1 as a ring-shaped univalent, which shows that iso-chromosomes are concerned here, but in *G. nutans* and *G. viminea* no evidence has been obtained to the effect that the supernumerary chromosomes are iso-chromosomes. In several cases it has been shown that the B chromosomes are of a heterochromatic nature. In the two *Godetia* species, however, the indications for heterochromatin are weak, though this also applies to the B chromosomes in rye.

The spindle attachment of the B chromosomes may have other properties than that of the A chromosomes. This may find expression in B univalents behaving differently from A univalents at meiosis. For instance, in *Anthoxanthum aristatum* (ÖSTERGREN, 1947) and *Poa alpina* (MÜNTZING, 1948) they pass undivided to the pole during anaphase 1, whereas A univalents are divided. This difference has an important consequence: unpaired B chromosomes seldom become eliminated during meiosis as do, on the other hand, unpaired A chromosomes. In *G. viminea* the supernumerary chromosomes are not divided and are rarely eliminated in spite of the fact that in most cases they lie on or near the equatorial plate at metaphase 1. However, as in *G. viminea* no difference in size can be observed between A and B univalents, it has been impossible to determine whether the latter have a lower elimination frequency than any possibly present A univalents. A univalents may be divided or remain undivided at anaphase 1, but the latter alternative does not exclude elimination here.

It is mainly certain peculiarities regarding the pairing of the supernumerary *viminea* chromosomes that seem to justify their being regarded as accessory chromosomes. As is known, a general rule is that B chromosomes do not pair with A chromosomes, and multivalent formation has not been observed either in *G. viminea* or in *G. nutans*. Another point is that B chromosomes may show a weak reciprocal pairing, although in this respect there are great differences in different

cases. Examples of B chromosomes that fairly regularly form bivalents are to be found in *Anthoxanthum aristatum* and *Poa alpina*, while *Secale* has B chromosomes that show very good pairing but also such with greatly reduced pairing. In maize the B chromosomes show reduced pairing. *G. nutans* presented plants with 30 chromosomes that always formed 15 II but also other such plants that almost always formed 14 II + 2 I. Certain results from progeny studies suggest that this dissimilarity was due to different pairing properties in different supernumerary *nutans* chromosomes. In *G. viminea* the supernumerary chromosomes were unpaired even when they were identical.

Formerly expression was often given to the view that B chromosomes are quite inert, but later investigations have shown that this is an exaggeration. Their activity, however, is considerably weaker than that of the A chromosomes. Further, B chromosomes generally have a tendency to remain in the progeny and not to become eliminated like supernumerary A chromosomes or chromosomes foreign to the species. ÖSTERGREN (1945) has called them parasitic chromosomes on account of their ability to keep their position in the population even if their effect is noxious. To this contributes the fact that in many cases they have acquired a special mechanism of multiplication. For instance, in *Secale* the B chromosomes undergo at the first division in the embryo-sac and pollen-grain a non-disjunction directed towards the generative pole. Hence, as a rule, egg-cells and male nuclei have an even number of B chromosomes and in consequence the majority of plants with B chromosomes have an even number of such. The last-mentioned is also the case in *Poa alpina*, though no process of non-disjunction has been discovered there. In certain cases non-disjunction of B chromosomes is only found on the male side, the result being that if a plant without B chromosomes is pollinated with pollen from another with 1 B chromosome there arise a number of plants with 2 B chromosomes, whereas this is not the case in the reciprocal cross. Crossing results of this kind have been obtained in *Anthoxanthum aristatum* (ÖSTERGREN, 1947) and in maize (see ROMAN, 1947). In the former case non-disjunction of the B chromosomes has been observed at the first pollen mitosis, whereas in maize it does not take place until at the second mitosis, when the generative nucleus divides with formation of two male nuclei (ROMAN, 1947): as a rule only one of the male nuclei has B chromosomes, but an even number of such, the other having none. This non-disjunction at the second pollen mitosis is followed by a directed fertilization (ROMAN, 1948). The fact is that in the majority of embryo-sacs the egg

nucleus is fertilized by the male nucleus that has B chromosomes while the polar nuclei are fertilized by the nucleus that has no B chromosomes. In maize the B chromosome is often lost during meiosis, but in spite of this B chromosomes very frequently occur in certain varieties of maize. This »could be accounted for if the mechanism of directed fertilization is more highly developed in these strains, as compared with others in which B-type is absent or rarely found» (ROMAN, *l. c.*).

Various mechanisms have thus been described as contributing to the persistence of B chromosomes in the population: little elimination of B univalents at meiosis, non-disjunction at the first division in the pollen-grain as well as possibly in the embryo-sac, non-disjunction at the second division in the pollen-grain followed by directed fertilization. In *Anthoxanthum* the first and second of these mechanisms are to be found, in *Secale* the second, in maize the third.

In *Godetia viminea* the supernumerary chromosomes show a low frequency of elimination in spite of the fact that during the first metaphase they always seem to behave as univalents. But, as already mentioned, it has not been possible to demonstrate that the frequency is lower than in the case of unpaired A chromosomes, and therefore it is still undecided whether the B chromosomes have a spindle attachment that differs from that of the A chromosomes. The only facts at our disposal by which to judge whether the supernumerary *viminea* chromosomes have a tendency to persist in the progeny and whether they possess any multiplying mechanism conditioned by non-disjunction are, accordingly, the chromosome counts in the progeny of two self-pollinated plants each having a supernumerary chromosome. As already mentioned, it is clear that the supernumerary chromosomes cannot be ordinary A chromosomes. On the other hand, the fact that most of the plants had 18 or 19 chromosomes shows that a regularly functioning mechanism of multiplication on the male and the female side cannot be present. But in both the progenies there were plants with 21 chromosomes, *i. e.* with too many chromosomes, which indicates that multiplication does take place though rather rarely. These plants have doubtless arisen from the fertilization 2 B + 1 B, which implies that the supernumerary chromosomes are inherited both through pollen and through egg-cells. As no reciprocal crosses with 18-chromosomal plants were examined, it is unknown whether it is the male or the female gamete which carries two B chromosomes, *i. e.* whether any non-disjunction may occur on the male or female side. Some of the 20-chromosome plants may also have arisen after non-disjunction, for

plants with two B chromosomes may have been produced through 1 B + 1 B or 2 B + 0 B fertilizations.

The chromosome numbers in the progeny of the self-fertilized 19-chromosomal plants thus show that the supernumerary chromosome is inherited both through male and females gametes and that it seems to undergo a multiplication, probably through non-disjunction, which, however, is not a constant process but only occurs rather sporadically. It is further evident that although both the progenies had many 18-chromosome plants the majority of plants had supernumerary chromosomes. These conditions suggest that the supernumerary *viminea* chromosomes possess some tendency to survive in the population in spite of their being unpaired at meiosis. Whether the supernumerary chromosomes have any effect on the vitality or fertility of the plants, is unknown, but it is certain that they have little or no effect on the appearance of the plants. They therefore seem to be relatively inert.

From all this the impression is obtained that there are accessory chromosomes in *Godetia viminea*, although not fully typical such. They are most closely reminiscent of the supernumerary chromosomes of *G. nutans* (HÅKANSSON, 1945), to which reference has already been made several times in this paper. Cytological conditions resembling those in these species appear to occur in *Ehrhartia calycina*, a polymorphic grass species that occurs in California (LOVE, 1948). LOVE found plants with 24, 25, 26, 27 and 28 chromosomes, but »approximately the same range of morphological variation occurred in all chromosome groups». Within all chromosome groups there also appeared albinotic seedlings. The supernumerary chromosomes do not pair with the normal chromosomes, although they often pair reciprocally (*cf.* certain plants of *Godetia nutans*). From LOVE's description it is further evident that the supernumerary *Ehrhartia* chromosomes are apparently of the same size as the ordinary chromosomes and that they produce a certain amount of asyndesis. LOVE's report is preliminary and he has not yet made comparisons with B chromosomes.

CAMERON (1934) found varying chromosome numbers in *Crepis syriaca*, a species closely allied to *C. alpina*. The normal chromosome number is $2n = 10$, but in addition 1—8 small chromosomes could be found of distinctive morphology. It is presumed that these new chromosomes had originated through chromosomal alterations in hybrids between two different *alpina* forms. CLAUSEN (1931) has found populations of *Viola canina* with very irregular cytology and up to 16—25 univalents. He considers (1932) that crosses between *V. canina* and

V. Riviniana take place, that »morphologically true and compatible populations of *Viola canina* established themselves as segregates from these crosses in habitats where the ecotype represented by *canina* is the preferred one, and that these populations were morphologically uniform before they were cytologically balanced». The cytological irregularities are preserved through intercrossings between different types. It was at first assumed that the supernumerary *viminea* chromosomes were derived from a species-cross, but their constant asyndesis is difficult to explain.

Some *viminea* plants had quite a different kind of supernumerary chromosomes. They were quite small fragments, which often seemed to become lost at divisions. Perhaps they have an ephemeral existence.

The discovery of a haploid *G. viminea* is a fresh example of the fact that haploids are rather easily formed within the *Godetia* genus, haploids having previously been found in *G. amoena* and *G. Whitneyi*. In *G. Whitneyi*, which has been subjected to extensive cytological investigations, rather many haploid plants have been discovered in the course of years. Most of the haploids have arisen after crosses between plants that must be regarded as rather unlike each other, but in *G. viminea* the haploid was found in the progeny of a self-fertilized 19-chromosomal plant. The haploid had no supernumerary chromosomes. Hence the embryo-sac from which it had arisen had not possessed any, but the male nucleus which presumably initiated the parthenogenetic development may have had such and, in consequence, between the gametes there may have been a difference that can have evoked or facilitated this development.

SUMMARY.

Godetia viminea has the chromosome number $2n = 18$. There are also plants with 19, 20 or 21 chromosomes. The supernumerary chromosomes have the same size as the ordinary ones. In the progeny of self-fertilized 19-chromosomal plants there were plants with 18, 19, 20 and 21 chromosomes. The commonest pairing in them was, respectively, 9 II, 9 II + 1 I, 9 II + 2 I and 9 II + 3 I. Hence, the supernumerary chromosomes were unpaired, although they must have been identically alike. There was also formed a haploid with 9 chromosomes. In a few 18-chromosomal plants some small fragment chromosomes were observed.

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ABSTRACTS — KURZE MITTEILUNGEN

ASKELL LÖVE: Agamospermy in *Acetosa*.

Although only relatively few of the many genera of the large family Polygonaceae have as yet been cytogenetically or embryologically investigated, occurrence of agamospermy has been observed in perhaps five or six genera as yet, *i. e. Oxyria* (EDMAN, 1929), *Atraphaxis* (EDMAN, 1931), *Rumex* (DUDGEON, 1918), *Acetosella* (MURBECK, *cf.* NORDSTEDT, 1907; LÖVE, 1943), and *Acetosa* (ROTH, 1906; LÖVE, 1942). Only in the genus *Atraphaxis* is the agamospermy obligate, and within that genus it is known to be of the type termed apospory (EDMAN, *l. c.*; *cf.* GUSTAFSSON, 1946, 1947). In *Oxyria* the observations made by EDMAN (*l. c.*) are not applicable to all ecotypes of the species, as at least some part of the material from the Scandinavian mountains and Iceland studied by the present writer certainly reproduce — at all events mainly — by sexual methods. Observations made by DUDGEON (*l. c.*) on *Rumex crispus* may also be somewhat dubious, although in the experiments performed by the present writer on this species some indications of the occurrence of at least facultative agamospermy in a low frequency of ovules have been obtained. In the arctic-alpine annual species *Koenigia islandica*, too, indications of the occurrence of agamospermy, perhaps of the aposporous type, are met with in Icelandic material as well as in material from the Scandinavian mountains fixed by Professor EDMAN and handed over to the present writer. This material, however, has not been completely studied as yet (LÖVE, unpubl.).

In the spring of 1939 the present writer began extensive investigations on the biological behaviour of different genera of Polygonaceae, especially the dioecious genera *Acetosella* and *Acetosa* and the Scandinavian representatives of the genus *Rumex s. str.* (for nomenclature, *cf.* LÖVE and LÖVE, 1948). The investigations were performed at the Institute of Genetics of the University of Lund, Sweden, mainly at its substation at Svalöf, during the years 1939—1945, but since then they have been worked out at our present institute in Reykjavík.

In 1942 some of the results obtained from the studies of the genus *Acetosa* were published in a short paper (LÖVE, 1942), and its taxonomical groups were described somewhat later on (LÖVE, 1944; LÖVE and LÖVE, 1948). In the paper of 1942 some comments were made as to the occurrence of agamospermy in the material of *Acetosa* from Scandinavia and Iceland, and it was mentioned that the agamospermy observed must be only of a facultative kind. In 123 of more than 300 isolations made some few seeds, in most cases only 1—10, but in some cases 20—25, were found to develop and give rise to plants, which were all found to have the diploid chromosome number $2n = 14$ and two *X* chromosomes. No plants had flowered when the paper was published in 1942, but the next summer all the plants flowered. They were all found to be female and strictly maternal in their appearance. The plants in question belonged to the species *Acetosa arifolia*, *A. pratensis*, and *A. thyrsiflora*. The highest frequency was met with in the plants belonging to *A. arifolia* from Iceland and northern Sweden (2,3 % in 53 % of the plants studied), but the lowest frequency was met with in *A. thyrsiflora* from Scania in southernmost Sweden (0,3 % in 21 % of the plants).

In the summers of 1941 and 1942 new isolations were made of female plants from different localities. Practically the same frequency of agamospermous seed setting was found in this material, too. One plant, however, formed an astonishing exception, as the three branches isolated were found to have a complete seed setting in the isolation bags as well as on the branches outside. This plant belonged to the species *A. arifolia* and originated from the neighbourhood of Reykjavík, and it looked just like normal female plants from the same locality.

Of course, the seeds obtained after isolation of the female plant in question were suspected to be the result only of an unsuccessful isolation, although no holes were detected in the bags. The seeds were grown out, however, and 200 plants were placed in the experimental fields at Svalöf in the spring of 1942. At the same time 100 plants obtained from seeds of unisolated branches of the same female plant were placed in the experimental fields in order to test their sexual and cytological behaviour compared with the first-mentioned progeny.

Only very few of the plants flowered during the summer of 1942, but in the summer of 1943 all the individuals flowered. At flowering time all these individuals were found to be of the same appearance, and not a single male plant was found in the progeny from the isolations or from the unisolated branches of the mother plant. All these 300 individuals were females of the Icelandic ecotype from Reykjavík, which is rather unlike the normal Swedish and Alpine ecotypes of the same species.

Although this evidence might be strong enough to indicate asexual seed setting of this plant and its progeny, the writer made fixations of all the plants in order to obtain embryological material as well as mitotic chromosomes for studies of their karyotype. Unfortunately, however, he has not been able to make complete analyses of the material until now, partly because he moved to Reykjavík and partly owing to lack of experimental fields and apparatus for microscopical work. In the fixations of the flowers no useful material was obtained for embryological analyses, but in the root tips of all the plants the chromosomes could be closely studied and measured. For the cytological methods used, *cf.* LÖVE (1943).

In the genus *Acetosa* subgenus *Dioeca* sectio *Euacetosae* all the male plants are found to have the diploid chromosome number $2n = 15$, three of which are sex chromosomes, 2 *Y* chromosomes and 1 *X* chromosome. The female plants, however, have only $2n = 14$ chromosomes with only two *X* chromosomes. The chromosomes within this group are rather large and their shape and form are not exactly the same in all individuals of the same species. Therefore, some so-called karyotypes have been described within some of the species (*cf.* KIHARA and YAMAMOTO, 1931; ONO, 1935; YAMAMOTO, 1933, 1938; LÖVE, 1942), and the different karyotypes are found to have a somewhat different distribution in Japan as well as in Scandinavia and Iceland.

The karyotype of the female plant from the neighbourhood of Reykjavík was found to be $10\,i + 2\,i^s + 2\,X$ chromosomes, and the i^s chromosomes were found to be the second smallest autosome pairs. Exactly this karyotype has not been mentioned by the Japanese workers, as the SAT chromosomes of the

almost corresponding karyotype in Japan are found to be the third longest autosome pair. For the signs of the karyotypes, *cf.* LÖVE (1943).

All the individuals belonging to the progenies of the above-mentioned female plant were found to have the chromosome number $2n = 14$, and in every case the karyotype was found to be the same, *i. e.* $10\,i + 2\,i^s + 2\,X$, or exactly the karyotype of the original mother plant.

The karyotype mentioned was also found in some other female individuals from the same locality, but those individuals were normal amphimictic females with a relatively high degree of facultative agamospermy (2,3—4,1 % seed setting after safe isolations). In order to test possible differences in length of some of the chromosomes in these individuals and the agamospermous progeny, the chromosomes from five plants of each group were carefully measured in the microscope. They were, however, not found to differ in any way, and therefore it might be suggested that the agamospermy observed is not caused by deletions of large or measurable parts of any of the chromosomes.

As mentioned above, no embryological preparations have as yet been studied. Therefore, it is impossible to decide at present what type of agamospermy is met with in the plants studied by the writer. All the results show, however, that in one of the Icelandic biotypes of *Acetosa arifolia* obligate agamospermy is met with, and that in a rather high frequency of the material of just this species from the same locality facultative agamospermy is met with besides the normal amphimictic seed formation. Therefore, it might be suggested that both these types of asexual seed setting are genically controlled and that the first plant with obligate agamospermy has been a result of an unusual accumulation of genes for agamospermous seed formation. This suggestion is supported by the relatively high frequency of facultative agamospermy in the material from the same locality.

Summary. — Agamospermous seed formation has been observed in material of the dioecious diploid species *Acetosa arifolia*, *A. pratensis*, and *A. thyrsiflora*. It is clearly facultative and occurs only in a low percentage of the ovules, highest in the northern material of *A. arifolia*, lowest in the more southern material of *A. thyrsiflora*.

In one Icelandic plant and its progeny the agamospermy was found to be absolutely obligate, as no seed was formed in the normal amphimictic way. This plant belonged to the Icelandic type of *A. arifolia* and originated from the neighbourhood of Reykjavík. Its karyotype was quite normal and of exactly the same type as that of the normal amphimictic females from the same locality. The frequency of facultative agamospermy among the other females from the same locality was unusually high.

It is suggested that the agamospermy met with in *Acetosa* is genically determined and that the obligate agamospermy of the female plant in question and its progeny is the result of an unusual accumulation of genes for this abnormal type of seed formation.

Agamospermy has at present been observed in the following genera of Polygonaceae: *Oxyria*, *Atraphaxis*, *Rumex*, *Acetosella*, *Acetosa*, and *Koenigia*,

although it has not been thoroughly studied in all cases and in most cases it is perhaps only facultative.

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BENGT KIHLMAN: The effect of purine derivatives on chromosomes.

It has been shown that caffeine and theophylline are able to induce mutations in the ascomycete *Ophiostoma multiannulatum* (FRIES and KIHLMAN, 1948) and chromosome changes in *Allium* (KIHLMAN and LEVAN, 1949). The research on the cytological effect was extended to include 15 purine derivatives. The test object was as before root-tips of *Allium Cepa*, kindly provided by Dr. ALBERT LEVAN of Lund.

Most of the purines have been synthezised by the author. The ethyl

xanthines only were obtained from the Research Laboratory of the N. V. Organon through the courtesy of Professor SUNE BERGSTRÖM, Lund.

The particular stress in this investigation is laid on the effect on the chromosomes. Other changes which the purines induce in the root meristem will only be briefly mentioned.

The toxic effect of the purines increases when methyl groups are substituted by other alkyl groups.

The allyl group is less toxic than the propyl group.

Purine derivatives	Primary effect: The lowest conc. tested where the effect is to be seen		Secondary effect	
	mmol	%	% AA/mmol purine	$\frac{\%\ \text{AA/mmol purine}}{\%\ \text{AA/mmol caffeine}} \times 100$
1,7-dimethylhypoxanthine	0	0	+	+
theophylline	+	+	1,0	33
caffeine	2	0,04	3,0	100
8-methoxycaffeine	0,2	0,004	4,8	160
8-ethoxycaffeine	0,2	0,005	6,2	207
8-propyloxycaffeine	0,2	0,005	3,3	110
8-allyloxycaffeine	0,2	0,005	6,0	200
1-ethyltheobromine	+	+	+	+
1,3-diethylxanthine	+	+	+	+
1,3,7-triethylxanthine	+	+	+	+
1,3,7-trimethyluric acid	+	+	+	+
1,3,7,9-tetramethyluric acid	0,5	0,01	19,5	650
9-ethyl-1,3,7-trimethyluric acid ..	5,0	0,12	2,2	73
9-allyl-1,3,7-trimethyluric acid ..	+	+	1,0	33
1,3,7,9-tetramethyl-4,5-dimethoxy-dihydrouric acid	0	0	0	0

+ means that primary or secondary changes, respectively, are too weak to be expressed in numerals.

% AA means the percentage of abnormal anaphases.

Later 8-chlorocaffeine was also tested. The secondary effect proved to be intermediary between the effect of the 8-ethers of caffeine and tetramethyluric acid, or, in numerals, 10 % AA/mmol, and 333 per cent of the caffeine effect. The threshold of the primary effect lies between 0,5—0,2 mmol (0,01—0,005 per cent).

In the same way the c-mitotic tendencies increase when methyl groups are substituted by other alkyl groups and the water solubility decreases. This fact is in agreement with observations made by LEVAN and ÖSTERGREN (1943).

In conformity to the methylxanthines the alkylated oxypurines so far investigated suppress the cell wall formation, although the effect is not always so obvious as, for instance, that of caffeine. Tetramethyl-4,5-dimethoxydihydrouric acid, which lacks the double bond between the carbon atoms 4 and 5, is however quite inactive.

The physiological or primary changes, *i. e.* the »stickiness» and »pseudochiasma» (LEVAN and TJIO, 1948) phenomena were analysed after a treatment of four hours. As appears from the accompanying table, tetramethyluric acid and the 8-ethers of caffeine induce primary chromosome changes in four to ten times more diluted solutions than caffeine. The stickiness phenomenon induced by these compounds is also of a more extreme type, and usually results in anaphase bridges between all of the separating chromosome arms. Tetramethyl-4,5-dimethoxydihydrouric acid is completely inactive, dimethylhypoxanthine and the ethylxanthines are practically inactive, whereas theophylline, 9-allyltrimethyluric acid and 1,3,7-trimethyluric acid induce a weak effect.

The structural or secondary chromosome changes were analysed after a treatment of 24 hours followed by 1—4 days in water. The slight solubility in water of the purine derivatives has sometimes rendered the investigation more difficult (*viz.* propyloxycaffeine, allyloxycaffeine) or impeeded it completely (theobromine).

The analysis is performed in such a way that the anaphases with structural chromosome changes are counted and expressed in per cent of the total number of anaphases on the slide. Each substance is tested in a series of concentrations and the percentage of abnormal anaphases is determined per mmol of purine. Since the effect is usually not proportional to the concentration, the highest value thus obtained is taken as an expression of the strength of the effect. As a comparison, the effect of each substance is then expressed in per cent of the caffeine effect calculated in the same way. This method of analysis is not exact, the standard error being about $\pm$ 10 %. In such a comparative investigation the method is suitable, however.

It appears from the table, that the 8-ethers of caffeine and especially tetramethyluric acid, again, are much more effective than caffeine, while tetramethyl-4,5-dimethoxydihydrouric acid is totally inactive, trimethyluric acid and dimethylhypoxanthine practically so.

When further comparing caffeine with 1-ethyltheobromine and with 1,3,7-triethylxanthine, theophylline with 1,3-diethylxanthine and finally tetramethyluric acid with 9-ethyltrimethyluric acid it will be seen that the substitution of a N-bound methyl group with an ethyl group considerably reduces the effect. When several methyl groups in this way are substituted by ethyl groups the purine derivative is practically inactive. On the other hand, it seems that the length of the carbon chain in the oxygen bound alkyl groups of the 8-ethers of caffeine does not in a higher degree influence the effect. The effect seems further to increase with an increasing N-methylation and with an increasing number of electronegative substituents in positions 6 and 8.

An explanation of these results is possibly to be found in an investigation by WEIL-MALHERBE (1946). He analysed the solvent effect of a great number of natural and synthetical purine derivatives on *int. al.* 3,4-benzpyrene. Among these purines are also such as have been tested in the present investigation. A comparison shows that a striking concordance exists between the solubilizing power, on the one hand, and the strength of the effect on the chromosomes, on the other. The only exception, 1,3,7-trimethyluric acid, may also be explained from the investigation by WEIL-MALHERBE, showing that purines with acidic functions are strongly inactivated when the acidic group is ionized. He

further points out that such purines in the buffered medium of the cell are likely to be ionized. Hence it is to be expected that they are inactive there.

So far as it can be estimated from the rather limited number of purine derivatives hitherto tested, the same rules are valid with regard to the solvent effect as well as to the cytological one. The latter is thus also favoured by those molecular-constitutional factors which, according to WEIL-MALHERBE, favour both the polarizability and the hydrophilic character of the molecule.

A detailed account of the results of the experiments will be published when the comparative investigation of the effect of purine derivatives on chromosomes and micro-organisms is finished.

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